Mitochondrial d-loop variation and DNA preservation in wild and domestic equids (*Equus sp.*) in Switzerland from the Palaeolithic to the Iron Age

Inauguraldissertation

zur

Erlangung der Würde einer Doktorin der Philosophie

vorgelegt der

Philosophisch-Naturwissenschaftlichen Fakultät

der Universität Basel

von

Elsner, Julia

aus Haldensleben, Deutschland

Kassel, 2018

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät

auf Antrag von Prof. Jörg Schibler, Dr. Angela Schlumbaum, Prof. Michael Hofreiter.

Basel, den 22.03.2016

Dekan

(Prof. Jörg Schibler)

Danksagung

Diese Dissertation ist Teil des SNF-Projektes “Genetic signatures in wild and domestic horses (*Equus sp.*) during 40,000 years BC in Switzerland in comparison with present-day horse breeds” (K-31K1_120528/1). Finanzielle Unterstützung erhielt ich zusätzlich durch die Freiwillige Akademische Gesellschaft Basel und die Nikolaus und Bertha Burckhardt-Bürgin-Stiftung.

Das Untersuchungsmaterial wurde freundlicherweise von den folgenden Institutionen und Personen zur Verfügung gestellt: Archäologische Bodenforschung Basel-Stadt (Gasfabrik, Riehen-Ausserberg), Naturhistorisches Museum Basel (Allschwil-Ziegelei, Birseck-Ermitage, Münchenstein-Steinbruch, Schalberghöhle), Archäologie und Museum Basel-Land (Brügglihöhle, Kohlerhöhle), Augusta Raurica, Kantonsarchäologie Aargau (Mumpf), Archäologischer Dienst Bern (Abri Neumühle, Twann-Bahnhof), Université de Neuchâtel (Hauterive-Champréveyres, Monruz), Kantonsarchäologie Schaffhausen (Kesslerloch, Schweizersbild), Kantonsarchäologie Solothurn (Rislisberg, Käsloch), Séction Archéologie Cantonal de Vaud (Le Mormont), Site et Musée Avenches (Aventicum) und J. Sedlmeier.

Ich möchte meiner Dissertationsleiterin Angela Schlumbaum und meinem Korreferenten Michael Hofreiter, sowie Tosso Leeb, Stefan Rieder und Jörg Schibler, die das ganze Projekt mitinitiiert haben, und meinen Koautorinnen Sabine Deschler-Erb und Barbara Stopp, sowie José Granado meinen Dank für ihre Kooperation und Unterstützung aussprechen. Herzlich bedanken möchte ich mich auch für die grosszügige fachliche und persönliche Unterstützung bei meinen Kolleginnen und Kollegen am IPNA, besonders bei Simone Häberle und Ben Jennings. Etliche Fachkollegen haben mich im Verlauf der Dissertation beraten und mich von ihrem theoretischen und praktischen Fachwissen und/oder Laboren teilhaben lassen, allen voran Ludovic Orlando (Universität Kopenhagen), nicht minder wertvoll (i. a. O.) Isabel Alves (Schweizer Institut für Bioinformatik, Lausanne), Christian Anderson (BayeSSC), Michaela Drögemüller (Universität Bern), Kim Dung Huynh (Universität Basel), Peter Forster (fluxus-engineering), Simon Ho (Universität Sydney), Arne Ludwig (Institut für Zoo- und Wildtierforschung, Berlin), Patrice Méniel (Universität Burgund), Werner Müller (Universität Neuchâtel), Moritz Muschik (Universität Bern), Markus Neuditschko (Agroscope), Pauline Nuviala (Universität Burgund), Andreas Papassotiropoulus (Universität Basel), Melanie Pruvost (Institut Jaques Monod, Paris), Hans-Peter Rusterholz (Universität Basel). Ich danke meiner Familie, ohne deren Rückhalt dies alles nicht möglich gewesen wäre.

Content

List of tables

List of figures

All images which were not originally designed for this dissertation are used with consent of the rights holder.

1. Introduction

Horses are a model organism to study evolution, they were a key species of the Pleistocene steppe and their domestication development is intricate. They are one of the most appreciated and popular species in the world. In my thesis *Mitochondrial d-loop variation and DNA preservation in wild and domestic equids (Equus sp.) in Switzerland from the Palaeolithic to the Iron Age* I want to trace the matrilinear development of wild horses in the region, from the earliest archaeological records of caballine equids in the Pleistocene to their local disappearance at the beginning of the Holocene, and prospect into the variation of the venerated domestic animal at the beginning of the Common Era. I will first introduce the relationship of man and horse with a focus on Switzerland. As the fate of a wild species largely depends on the condition of the environment, an overview of the climatic and vegetation change in the Jura and the Alpine foreland during the last 50,000 years will be given. I will then outline the genetic loci investigated in this study, involving the mitochondrial displacement loop and several markers of the nuclear genome including coat colour and molecular sex markers. In chapter two, the research papers which resulted from these investigations are presented, followed by additional publications of thesis-related research. The main results are summed up in chapter four and augmented by further explorations of selected issues. The findings about Swiss wild and domestic horses are contextualised with published studies on Eurasian horses. In chapter five the thesis will be briefly recapitulated and closed with concluding remarks.

1.1 The relationship of man and horse in Switzerland

The horse has been one of the most important animals to humans since the Pleistocene when Palaeolithic hunters dispersed over Eurasia and exploited horses for meat (Olsen, 2006). Their beauty and power, also, were apparently so impressive to people that the horse is the most frequently represented animal in European Upper Palaeolithic cave and portable art (Reflexion group on Palaeolithic rock art, 1993) (figure 1). The majority of paintings date to the Magdalenian period, c. 20 to 13 thousand years before present (ka BP) (Leesch, 1993, Newell, 2009) when, however, reindeer was the primary focus of big game hunting. Towards the end of the Pleistocene *Equus ferus caballus*, Linnaeus 1758, populations rapidly declined in number and distribution in Eurasia; they even went extinct in North America (Haile *et al.*, 2009), and only persisted in forest-free pockets of Europe, and particularly in the steppe region between the Carpathians and Kazakhstan (Anthony, 2007, 196, Sommer *et al.*, 2011). The closely related *E. f. przewalskii*, Poljakow 1881, inhabited Asia's steppes further east.

Figure 1: Engravings of wild horse on bone fragment, Brassempouy, Grotte du Pape, France (left); (Picture by Henry Fairfield Osborn) and Schweizersbild, Switzerland (right); © Schweizerisches Nationalmuseum.

The first remains of *E. f. caballus* in Switzerland stem from palaeontologic, i.e. non-anthropogenic, contexts (Schmid, 1967). They date between c. 50 and 40 ka BP. Horses as human prey are first documented from a cave (Kohlerhöhle) in a Badegoulian context from the Northern Swiss Jura region near Basel dating to around 23 ka BP – time when the Jura glacier was maximally extended to about 50 km beeline of the site (Ivy-Ochs *et al.*, 2004). Horses are abundant amongst Magdalenian faunal assemblages and were probably widely dispersed in the open steppe landscape of the Swiss Plateau.

It is not understood when the decline of the population, inferred from decreasing find densities in Azilian and a complete absence of finds from the Mesolithic, began and whether it was initialised by human hunting pressure or the temperature rise at c. 14.7 ka BP which was followed by marked vegetation changes. Very few horse remains are noted from

Neolithic sites; those which do occur are very small and mainly appear in archaeological layers with an increased proportion of wild animals (Schibler *et al.*, 1997). This argues in favour of their status as wild animals, but it cannot ultimately preclude that they were first domestics.

It has been argued that the horse might have gone extinct entirely if it were not domesticated in the Eurasian steppes, either because climate change led to their nutrition base – open grassland interspersed with shrubs – shrinking or competition with domestic herds intensified (Budiansky, 1997, Olsen, 2006). Archaeozoologically, the beginnings of horse domestication are challenging to pinpoint because unlike other domestic species such as cattle, ovicaprines and pigs, horses did not decline in size and the typical kill-off pattern of livestock raised for dietary purposes, characterised by young males and older females, is challenging to verify or not apparent (Anthony, 2007). To date, however, various disciplines hold evidence for domestication in the region north of the Black- and Caspian Sea at least by 3 ka BC (Anthony, 2007, Ludwig *et al.*, 2009, Outram *et al.*, 2009, Warmuth *et al.*, 2012). It is indeed the multiple uses of the horse that made this animal increasingly valuable for humans. It provided meat and milk, but the use for riding, packing and pulling vehicles became much appreciated, as was the horse´s significance in warfare.

Figure 2: Bridle fragment made from deer antler, East European type, Early Bronze Age. From Toos-Waldi, Schönholzerswilen, Thurgau. Picture by Daniel Steiner, Amt für Archäologie Thurgau.

It is generally established that from Bronze Age onwards, horses in Switzerland are domestics when bridle fragments point to horse harnessing (figure 2).They came alongside trade routes from the East which became increasingly important in the metal ages. Unlike in Eastern Europe and Central Asia, horse husbandry in Switzerland was mainly centred around the exploitation of the animals' work power and less around meat and milk production (Müller-Lhotska, 1984, 98). The horse, and horse-donkey hybrids (mules), were particularly relevant in trans-Alpine transport. It remained, however, rare; only as it gained military significance in the Iron Age numbers grew – but not to ubiquity; husbandry was reserved to the Celtic aristocracy. The horses had become even smaller since the Neolithic and ranged from 110 cm to 130 cm withers height. The rarely encountered larger horses (> 140 cm) are often referred to as imports from Rome yet it is possible that individual equids had been raised locally for representation.

The Romans bred horses for different purposes, such as traction, courier service, circus performance, chariot race and riding. The horse was, in contrary to the peace-loving other

domestics, described as war-loving (Vergil, Georgica, III.83-85: Erren, 1985-2003). Since the horse is a flight animal it has to be thoroughly trained to stand in battle. Nevertheless it was a man-made weapon in antiquity and gained its prestige from its deployment in war (Junkelmann, 1991, 13). In fact, no other animal has had such a tremendous impact on geopolitics (Olsen, 2006), for instance through the Roman (auxiliary) cavalry or the notorious Huns who conquered large parts of Eurasia on horseback in the fourth and fifth centuries AD, stimulating the Great Migration and contributing to the collapse of the Roman Empire (Kelly, 2008). It is said that "A dog may be a man's best friend, but the horse wrote history." (Unknown author). Likewise in Medieval times the horse is mainly associated with aristocracy, namely knighthood. The consumption of horse meat was prohibited by the Catholic Church as it was deemed unchristian. As the demand for horses enhanced prices, the first commercial stud farms were established. The oldest still existing stud farm in Europe is Einsiedeln Abbey, canton Schwyz, and was first noted in 1064 by King Henry IV (Huber, 1963); the local breed Einsiedler originates here. It is not until early modern history that horses started to become common. Agriculture, transportation of goods and passengers, and the military would have been unthinkable without horsepower into the 20th century. Yet, gradually the horse was replaced by machines. In Switzerland this led to a drastic decline of indigenous stock. The Einsiedler lost their status as a separate breed, and in order to conserve the indigenous Franches-Montagnes (figure 3) the Federal (later National) Stud Farm was established in 1898 in Avenches, canton Vaude (Nationalgestüt Avenches *et al.*, 2004, Rieder, 2015). During recent decades the public interest in local varieties of domestic animals has increased. Equid husbandry grew again in Switzerland, and horses are popular sport and leisure animals (Poncet *et al.*, 2007, Poncet *et al.*, 2009), indeed they have regained their status as the most expensive and valued domestic animal.

Figure 3: Franches-Montagnes in pasture on the National Stud Farm in Avenches. Pictures by Martin Rindlisbacher, SNG.

1.2 Environmental change in the Jura and Alpine foreland from 50,000 years ago until the present

The heterogeneous landscape between and including the Alps and the Jura Mountain chain – present day Switzerland – has been subjected to climate induced alterations. Several approaches serve to reconstruct climate change: the biological examination of plant micro- and macro-remains, molluscs and insects; the investigation of varves, sedimentation and weathering with lithogenic methods; geomorphological ascertainment and evaluation of moraines and other erosion- and accumulation-phenomena; as well as the physical-chemical analysis of oxygen isotopes. The results can be used to contextualise population developments of animals and humans. Recently, the various documentations of temperature, vegetation and ecology have been compiled and supplemented for the time frame of 60 to 8 ka BP for the Alpine foreland (Heiri *et al.*, 2014) and 40 to 8 ka BP for the Jura Mountains (Cupillard *et al.*, 2015). Environmental data from the time preceding the Last Glacial Maximum (LGM), i.e. before 30 ka BP, is notoriously sparse and discontinuous from the Jura Mountains and the Swiss Plateau, because most of the evidence was eroded by glacier movement. However, a few sites provide well dated key records, e.g. Niederwenigen, canton Zurich, and Gossau, canton Sankt Gallen. The peat of Niederwenigen was deposited c. 45 ka BP (Hajdas *et al.*, 2007, Preusser *et al.*, 2007) when mean temperatures ranged from 8 and 13 °C in summer to -20 and -5 °C in winter, and the landscape was characterized by open spruce (*Picea*)-pine (*Pinus*) forest as reconstructed from beetle remains, plant macrofossils and pollen (Coope, 2007, Drescher-Schneider *et al.*, 2007). The sediment record from the gravel pit in Gossau, constrained to c. 60-30 ka BP (Preusser *et al.*, 2003) revealed a similar picture. The older layer was formed in mean temperatures of 12 to 13 °C (summer) and -15 to -7 °C (winter) surrounded by open *Picea-Pinus* forest; the younger layer suggests open pine forest and colder mean temperatures between 8 and 12 °C in summer and -21 and -14 °C in winter (Jost-Stauffer *et al.*, 2001, Jost-Stauffer *et al.*, 2005). In general the climate was much more continental than today.

In the Swabian Alb, approximately 140 km to the north, the landscape was apparently more opened compared to Niederwenigen and Gossau. The analysis of plant micro- and macrofossils from the Upper Palaeolithic cave site Hohle Fels gives insight into the sequential vegetation development and human plant use in and around the cave from the Aurignacian to the Gravettian period (c. 44 – 32 ka BP) (Riehl *et al.*, 2014). Prevailing shrub tundra with cold steppe elements and only single woody patches is evidenced from the Aurignacian and Gravettian horizons, the following occupation hiatus is coincidental with the cold stadial between 25 and 15 ka BP.

Figure 4: Switzerland during the last Glacial Maximum. Source: Swiss Federal Office of Topography.

The Jura ice sheets reached their maxima between c. 27.5 and 24 ka BP (Buoncristiani *et al.*, 2004) and the Alpine LGM is dated to 23 to 19 ka BP in the Swiss Plateau (Ivy-Ochs *et al.*, 2008, Preusser *et al.*, 2011) (figure 4). The northern slopes of the Jura remained ice-free. The carrying capacity of the vegetation was apparently sufficient to feed large herbivores and allow for human (temporary) settlement as indicated by faunal remains in Badegoulian contexts from a few cave sites (Terberger *et al.*, 2001, Sedlmeier, 2010). The Swiss Plateau and Jura region were ice-free by at least 17.5 ka BP, and in succession were rapidly vegetated by cold-resistant and heliophile grasses and herbs, before eventually being populated by herbivores and humans (Leesch *et al.*, 2012b). The Oldest Dryas pollen records from Jura lakes, mires, bogs and marshes are dominated by Poaceae (true grasses), *Artemisia* (e.g. common mugwort) and pine; *Pinus*-values below 20 % are interpreted as resulting from long-distance transport (Cupillard *et al.*, 2015). The contemporary lake sediments and peat deposits from the lowlands also indirectly support the picture of an open herbaceous landscape, as datable organic remains are absent prior to afforestation after 14.7 ka BP (Heiri *et al.*, 2014). The following Bølling is marked by an abrupt increase in summer temperature around 14.7 ka BP by c. 5 °C (Heiri *et al.*, 2014) initializing the rapid decline of *Artemisia*, Poaceae, Cyperaceae (sedges) and *Helianthemum* (sunrose) and the thrive of shrub flora. *Pinus* experienced a certain lag, yet flourished by c. 13,750 BP, the onset of the Allerød (Ammann *et al.*, 2013); the landscape changed from tundra to open woodland. There seems to be a shift in human occupation pattern away from the (foot of the) mountains into the plains (Nielsen, 2009) and a diversification of hunted prey when new species such as deer (*Cervus elaphus*) spread (Cupillard *et al.*, 2015). After another colder phase in the Younger Dryas around 12.7

ka BP, temperature rose again at the beginning of the Holocene c. 11.7 ka BP (Heiri *et al.*, 2014), climate and vegetation converged to present day conditions.

The Mesolithic is characterized by canopy deciduous forest as indicated by depleted δ^{13} C values for *C. elaphus* (Drucker *et al.*, 2011). By at least 7 ka BP, the Neolithic was established in Switzerland, as visible in an increase of "agricultural practices" in lacustrine and peat-bog pollen sequences from the Jura Mountains (Cupillard *et al.*, 2015), and this results in the initial dilution of the interdependence of latitude/temperature and vegetation. Colder and warmer periods are indicated by transgression and regression phases in the lake levels (Jacomet *et al.*, 1995). Temperature fluctuation also occurs in later periods, but generally they do not exceed 2 °C in the annual average (Magny *et al.*, 1998). The Swiss Plateau and Jura are widely covered with beech (*Fagus*), fir (*Abies*) and oak (*Quercus*) forest from Neolithic until the Medieval era, with deciduous trees dominating in the lowlands and conifers in higher altitudes (Richoz *et al.*, 1995, Jacomet *et al.*, 1999, 101, Wick, 2002, Brombacher *et al.*, 2005). The landscape was subsequently opened to meet the increasing demand for pasture, tillage and wood for construction and fire (Rachoud-Schneider *et al.*, 1998, Wick *et al.*, 2002), in Roman times intensely exploited areas were interspersed with dense forest (Hüster-Plogmann *et al.*, 2005). Today, about 30 % of Switzerland is forested. The Alps are dominated by coniferous and the Plateau by deciduous forest. Approximately 35 % is used for agriculture, 8 % is populated and 25 % unused (Bundesamt für Raumentwicklung ARE). The climate is temperate, sub-oceanic/semi-continental north of the Alps with mean annual temperatures from 7 to 9 °C (Bundesamt für Meteorologie und Klimatologie MeteoSchweiz).

1.3 Genetic loci investigated: Mitochondrial displacement loop, Single Nucleotide Polymorphisms and *STX17*

In recent years sequencing technique and computer processing development have advanced immensely; thus, molecular genetic studies are no longer limited to short segments of the genome. In fact whole genome sequencing has become such a routine it will be applied even when scientists are interested in particular genes in specific chromosome regions (Bailey, 2015). High throughput sequencing is also increasingly applied to archaeological material, and whole or draft genomes of microbial pathogens, humans, mammoth, equids and other organisms have been published (Orlando *et al.*, 2015). However, for processing large numbers of specimens the choice of specific targets is favourable. The choice of the target depends on the aims of the study, as some genetic loci are suitable for phylogenetic or population genetic approaches, and others for individual characterisations because of their different mutation rates. Variation in regions with lower mutation rate indicates identity by descent and not identity by state. Concerning ancient DNA (aDNA) preservation capacity and information value of short fragments have to be considered. Non-coding regions generally accumulate more polymorphisms due to reduced selection pressure. This includes, for example, the mitochondrial (mt) genome whose substitution rate is elevated by an order of magnitude compared to the nuclear (nc) genome, and in particular the mt displacement loop (d-loop). Mitochondria are cytoplasmic organelles in which the energy-generating process of oxidative phosphorylisation takes place (Jobling *et al.*, 2004, 39). The double stranded circular mt genome spans between 15 and 20 k bases. There are a number of possible reasons for the elevated mutation rate; e.g. the mitochondrion contains high concentrations of mutagenic oxygen free radicals, it is not packed with histones and the mt replication system includes a higher turnover rate, longer duration of single-strandedness and less effective repair (Jobling *et al.*, 2004, 61). Mitochondria occur in high copy numbers in each cell and are inherited maternally only, and thus provide advantages for aDNA approaches: a high number enhances the chance of survival in degraded tissue and the absence of recombination allows the tracking of matrilineages over many generations. The analysis of ancient mtDNA (d-loop) variation has been utilised to address questions concerning phylogeny (Barnett *et al.*, 2009, Willerslev *et al.*, 2009, Fu *et al.*, 2013, Vilstrup *et al.*, 2013), animal domestication (e.g. Fernandez *et al.*, 2005, Edwards *et al.*, 2007, Larson *et al.*, 2007), migration (e.g. Larson *et al.*, 2010, Brandt *et al.*, 2013, Meiri *et al.*, 2014) and population development (e.g. Campos *et al.*, 2010b, Lorenzen *et al.*, 2011, Brace *et al.*, 2012, Welch *et al.*, 2012, Stiller *et al.*, 2014, Scheu *et al.*, 2015).

However, information about individual characteristics such as phenotype and sex can only be retrieved from coding DNA segments on the autosomes and allosomes. Most common are simple base-substitutional differences called single nucleotide polymorphisms (SNP), this category also includes small insertions and deletions (indels). Within the open reading frame (ORF) they have a range of effects – from silence (no effect) to complete abolition of protein production; and gene expression can even be affected by SNP outside the ORF (Jobling *et al.*, 2004, 56). Most mutations concerning phenotype are disadvantageous as there are more ways to impair the function of a gene than to enhance it, thus, for example, particular coat colourations are accompanied with pathologic pleiotropies. Melanocytes (pigment cells) are

found in the skin, hair, eyes, inner ear and leptomeninges. They are derived from embryonic cells of neural crest origin which also give rise to bone, cartilage, adipose, endocrine cells and several types of neurons and glia (Le Douarin *et al.*, 1999, 2). The causation of a malfunctioning melanin synthesis or integration is thus likely to affect these domains as well and may for example lead to hearing, visual or metabolic deficits which can even be lethal.

With horses, the mentioned impairments are mainly associated with (partially) white coat colourations (Reissmann, 2009, 20, Bellone, 2010). It is not surprising that these only emerge in noticeable frequencies in the course of domestication when selection mechanisms are indeed reversed in favour of "interesting" colourations (Ludwig *et al.*, 2009). An exception is leopard complex spotting which occurred in wild horses and apparently provided a selective advantage in the glacial steppe landscape despite the congenital stationary night-blindness in homozygous individuals (Ludwig *et al.*, 2015). The most prevalent variant of white coat is grey. Grey horses are born with any original colour and turn white over time due to premature progressive depigmentation. This development is caused by a 4.6 k base duplication in intron 6 in the *STX17* gene (Syntaxin-17) on chromosome 25 (Rosengren Pielberg *et al.*, 2008). The amplification of such a long fragment is challenging concerning aDNA – compared to the relatively easier accessible SNPs – yet it is sufficient to target the region spanning the end of the first 4.6 k base segment and the beginning of the duplication. To authenticate the outcome of this test, the region spanning the end of the segment and the beginning of the sequel of intron 6 should be targeted as well. The SNPs associated with the basic colourings bay, chestnut and black, the dilutions cream and silver and the spotting patterns overo, tobiano and sabino were investigated for the dissertation, focussing on Iron Age and Roman time horses. These samples also underwent a molecular sexing routine using sex-specific markers within the amelogenin- and zinc-finger-structures on the allosomes (Lippold *et al.*, 2011a ; Research Paper Three).

The investigation of coat, mane/tail, or feather and skin colouration in archaeological remains is fascinating. To identify the ratio of older and younger females and males allows the recognition of specific kill-off patterns associated with domestication. We can understand domestication processes and the changes it involved in animal behaviour and phenotype (Trut *et al.*, 2009, Girdland Flink *et al.*, 2014, Schubert *et al.*, 2014) and we can identify (early) domestics (Ludwig *et al.*, 2009, Krause-Kyora *et al.*, 2013), and thus draw inferences on people's knowledge and behaviour at the time. Moreover, we can reconcile prehistoric and historic depictions and descriptions of animals (Rawlence *et al.*, 2009, Campana *et al.*, 2010, Pruvost *et al.*, 2011, Svensson *et al.*, 2012, Svensson *et al.*, 2014, Zouganelis *et al.*, 2014, Imsland *et al.*, 2016), and not least, contribute to a literal picture of the past that often has to remain very vaguely sketched in archaeology (Fortes *et al.*, 2013).

1.4 Aim and prospect of the thesis

The thesis *Mitochondrial d-loop variation and DNA preservation in wild and domestic equids (Equus sp.) in Switzerland from the Palaeolithic to the Iron Age* is part of the SNF project K-31K1_120528/1 *Genetic signatures in wild and domestic horses (*Equus *sp.) during 40,000 years BC in comparison with present-day horse breeds*. The general aim of the project was a chronological genetic investigation of archaeological horse remains from Switzerland including mitochondrial d-loop variation and coat colour identification, and the exploration of the genetic diversity of rare extant breeds. In this thesis, I investigated the sites presented in figure 5, focussing on three main subjects.

Firstly, mtDNA preservation of Pleistocene equid remains in the context of different burial conditions. To date, specimens from waterlogged preservation conditions (Schlumbaum *et al.*, 1997, Schlumbaum *et al.*, 1998, Pollmann *et al.*, 2005, Bernicchia *et al.*, 2006, Larson *et al.*, 2007, Pruvost *et al.*, 2008, Schlumbaum *et al.*, 2011, Schlumbaum *et al.*, 2012, Schibler *et al.*, 2014), dry sites (Lassen *et al.*, 2000, Schlumbaum *et al.*, 2003, Schlumbaum *et al.*, 2006, Larson *et al.*, 2007, Pruvost *et al.*, 2008, Warnberg *et al.*, 2012, Haas *et al.*, 2013, Svensson *et al.*, 2014) and even permafrost situations (Schlumbaum *et al.*, 2010) from Switzerland have been targeted – not always successfully. A systematic synthesis of the influence of different burial conditions on DNA amplification success is still outstanding, here, however, the problem is addressed concerning Pleistocene equid teeth and bones, excluding permafrost preservation but augmenting the spectrum of depositional contexts by cave sites. Additionally, Neolithic, Iron Age and Roman horse remains from dry and waterlogged archaeological sites were compared concerning DNA amplification success and amount of post mortem damage derived lesions.

Secondly, mtDNA d-loop variation of Pleistocene and Early Holocene wild horse populations in Switzerland is investigated. Recent compilations of palaeoclimatological, palaeoenvironmental and archaeological data (Heiri *et al.*, 2014, Cupillard *et al.*, 2015) allow the relation of the development of equid population demography to the Alpine and Jura LGM, afforestation following atmospheric temperature increase by 14.7 ka BP or alterations of the landscape in the course of human agricultural activities. This is the first comprehensive survey of all known Pleistocene equid remains from a restricted region and is thus apt to demonstrate demographic patterns in reaction to a changing environment and human encroachment that might be overlooked in global studies (Weinstock *et al.*, 2005, Cieslak *et al.*, 2010, Lorenzen *et al.*, 2011, Orlando *et al.*, 2013). The results of this analysis are compared to the demographic development of Pleistocene horse populations from northern Asia (Taymyr Peninsula and Sakha Republic, Russian Federation) and the Urals region.

Thirdly, we examine mtDNA d-loop variation coat colour and sex of Iron Age domestic horses. Archaeozoological studies have defined a standard Celtic horse which is small, with withers height between c. 110 to 130 cm (Peters, 1998, Arbogast *et al.*, 2002). The rarely occurring larger horses (> 140 cm) are regarded as Roman introductions because Roman livestock breeders raised larger domestics, and this trend continues in the regions that are subsequently added to the Roman Empire. However, direct biomolecular evidence for the trade/exchange of equids is presently scarce (Paulus *et al.*, 2007, Nuviala *et al.*, 2014). A

possible genetic differentiation of morphologically different animals is enquired via matrilinear diversity, and the investigation of coat colouration and sex serves to detect (phenotypical) noticeable individuals and to relate their incidence to the archaeological context. This is the first study focussing Celtic horses, renowned for their endurance and fearlessness.

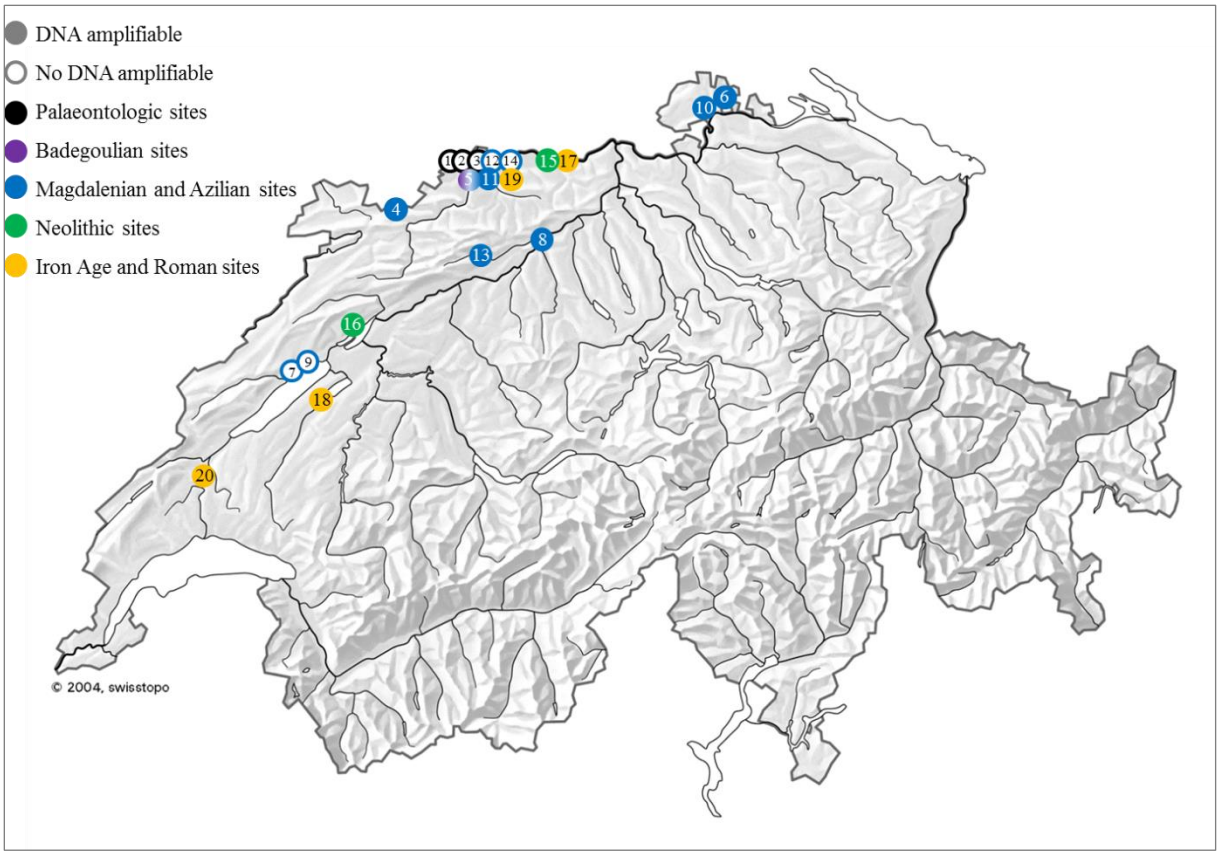


Figure 5: Investigated sites. 1 – Riehen-Ausserberg; 2 – Münchenstein-Steinbruch; 3 – Allschwil-Ziegelei; 4 – Schalberghöhle; 5 – Kohlerhöhle; 6 – Kesslerloch; 7 – Hauterive-Champréveyres; 8 – Käsloch; 9 – Monruz; 10 – Schweizersbild; 11 – Abri Neumühle; 12 – Birseck-Ermitage; 13 – Rislisberghöhle; 14 – Brügglihöhle; 15 – Mumpf; 16 – Twann-Bahnhof; 17 – Augusta Raurica; 18 – Aventicum; 19 – Basel-Gasfabrik; 20 – Mormont.

2. Research Papers

2.1 Elsner J, Schibler J, Hofreiter M, Schlumbaum A (2015) Burial condition is the most important factor for mtDNA amplification success in Palaeolithic equid remains from the Alpine foreland. *Archaeol Anthropol Sci* 7(4): 505-515, doi: 10.1007/s12520-014-0213-4

Archaeol Anthropol Sci
DOI 10.1007/s12520-014-0213-4

ORIGINAL PAPER

Burial condition is the most important factor for mtDNA PCR amplification success in Palaeolithic equid remains from the Alpine foreland

Julia Elsner · Jörg Schibler · Michael Hofreiter · Angela Schlumbaum

Received: 13 November 2013 / Accepted: 22 September 2014

Abstract Faunal remains from Palaeolithic sites are important genetic sources to study preglacial and postglacial populations and to investigate the effect of climate change and human impact. Post mortem decay, resulting in fragmented and chemically modified DNA, is a key obstacle in ancient DNA analyses. In the absence of reliable methods to determine the presence of endogenous DNA in sub-fossil samples, temporal and spatial surveys of DNA survival on a regional scale may help to estimate the potential of faunal remains from a given time period and region. We therefore investigated PCR amplification success, PCR performance and post mortem damage in c. 47,000 to c. 12,000-year-old horse remains from 14 Palaeolithic sites along the Swiss Jura Mountains in relation to depositional context, tissue type, storage time and age, potentially influencing DNA preservation. The targeted 75 base pair mitochondrial DNA fragment could be amplified solely from equid remains from caves and not from any of the open dry and (temporary) wetland sites. Whether teeth are better than bones cannot be ultimately decided; however, both storage time after excavation and age significantly affect PCR amplification and performance, albeit not in a linear way. This is best explained by the—inevitable—heterogeneity of the data set. The extent of post mortem damage is not related to any of the potential impact factors. The results encourage comprehensive investigations of Palaeolithic cave sites, even from temperate regions.

Keywords Ancient DNA · DNA preservation · Horse · Cave · Switzerland

Introduction

The analysis of ancient DNA from archaeological specimens provides the unique opportunity to study genetic diversity at different time intervals of the past. Because of this, it is coveted not only in archaeology but also in other disciplines such as evolutionary, population and conservation genetics. However, progressive post mortem degradation limits the access to genetic information from sub-fossil material. The mechanisms of DNA decay, resulting in small amounts of highly fragmented and chemically modified molecules, have been intensively studied (e.g. Pääbo 1989; Lindahl 1993; Höss 1995; Hofreiter et al. 2001; Mitchell et al. 2005; Deagle et al. 2006; Hansen et al. 2006; Briggs et al. 2007; Brotherton et al. 2007; Gilbert et al. 2007; Vives et al. 2008; Lamers et al. 2009; Heyn et al. 2010; Allentoft et al. 2012; Overballe-Petersen et al. 2012; Dabney et al. 2013). Depending on burial and exposure temperature, rapid or slow sedimentation, chemical properties of the soil, pH value, the presence or absence of oxygen, water, ionic radiation and microorganisms, organic material is destroyed sooner or later. Under the most favourable conditions, such as ice cores or permafrost soil, short fragments of DNA can survive presumably up to one million years (Geigl 2002; Willerslev et al. 2007; Dabney et al. 2013; Orlando et al. 2013; Meyer et al. 2014). Yet, DNA preservation can be different in similar environments (Allentoft et al. 2012; Olalde et al. 2014), even in seemingly optimal permafrost (Campos et al. 2010). However, those environments hardly represent typical burial

Electronic supplementary material The online version of this article (doi:10.1007/s12520-014-0213-4) contains supplementary material, which is available to authorized users.

J. Elsner (✉) · J. Schibler · A. Schlumbaum
Integrative Prehistory and Archaeological Science, University of Basel, Spalenring 145, 4055 Basel, Switzerland
e-mail: Julia.Elsner@unibas.ch

M. Hofreiter
Institute for Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Str. 24-25, 14476 Potsdam, Germany

Published online: 04 October 2014

conditions. Large amounts of archaeobiological specimens from cultural layers are preserved in temperate climates. The prediction of DNA survival is difficult, and yet, as all archaeobiological remains are potentially valuable genetic resources of the past, archaeologists and museum curators need to optimally select precious samples for invasive analyses. At the same time, aDNA studies are costly—not only in terms of damaging unique samples but also in terms of labour and money—and thus, it is important to estimate the feasibility of a genetic study in a given region. Several parameters have been proposed as proxies for DNA survival, for example histology (Guarino et al. 2000), biochemical preservation, in particular amino acid racemization (Poinar et al. 1996) and the so-called thermal history (Smith et al. 2001). No consistent correlation was detected between any of the proposed proxies and DNA preservation (Götherström et al. 2002; Haynes et al. 2002; Rollo et al. 2002; Gilbert et al. 2006; Collins et al. 2009; Schwarz et al. 2009; Hoke et al. 2011). However, it has been shown that DNA degrades somewhat predictably, although with large variance, over time (Allentoft et al. 2012). Moreover, it has been suggested that burial condition substantially influences DNA preservation (Molak and Ho 2011).

Next generation sequencing (NGS) techniques can process short fragments of DNA and are thus particularly suitable for aDNA research. Some of the disadvantages of aDNA like limited amount of highly fragmented template (mainly <80 base pairs (bp); Pääbo et al. 2004; Deagle et al. 2006; Allentoft et al. 2012; Sawyer et al. 2012) could be overcome with these technologies, but the high costs and substantial labour input prior to and after NGS make it even more desirable to select suitable specimens. Conventional PCR amplification followed by Sanger sequencing is not only used to initially screen samples but we also expect that it will remain an important approach for answering many archaeologically relevant questions, such as species determination, identification of individuals and the relations between them, lineage history and genetic diversity. Large public databases for reference exist (e.g. GenBank), and there are sophisticated statistical routines at hand to evaluate the results of genetic analyses, even for short sequences (e.g. Anderson et al. 2005; Excoffier and Lischer 2010; Drummond et al. 2012). However, publicly accessible databases on DNA survival in relation to environmental, geological or depositional conditions are still limited and require further data input (e.g. http://thermal-age.eu/). Thus, comprehensive regional studies (e.g. Gravlund et al. 2012) and chronological surveys of aDNA preservation represent tools for local archaeologists or museum curators to select promising samples.

In this paper, we investigate the preservation of mitochondrial DNA (mtDNA) and post mortem damage in 182 archaeological horse remains ranging in age from c. 47 to c. 12,000 years (ky) from different depositional contexts in the temperate, sub-oceanic/semi-continental climate of Switzerland, typical for large areas in Europe. The 14 Palaeolithic sites are scattered along the Jura Mountains. They comprise all known locations with at least one equid remain from this time period. Detailed archaeological and depositional information is available for all specimens. We expected the degree of DNA preservation and post mortem damage-derived lesions to differ depending on burial context, tissue type, storage time after excavation, and sample age and assessed DNA preservation by polymerase chain reaction (PCR). The time frame investigated is interesting for genetic studies because it includes climatic events like the European last glacial maximum (LGM) ~23 to 19 ky BP (Hughes et al. 2013) and the subsequent warming which led to dramatic changes in the vegetation, as well as cultural developments like the arrival of anatomically modern humans in Europe and the spread of Magdalenian horse hunters, episodes that are likely to have shaped the structure of horse populations (Lorenzen et al. 2011; Orlando et al. 2013).

Material and methods

Archaeological samples

All samples were excavated in or close to the Jura Mountains, a limestone formation that folded up about 10 to 2 million years ago from Jurassic sediments. Today, the annual mean temperature in the area is ~10 °C with an average seasonal variation from ~0 to ~19 °C (www.meteoschweiz.admin.ch). A total of 182 horse (*Equus* sp.) teeth and bones were taken from three types of sites: (i) Caves (with a maximal depth of 15 m) and rock shelters (abris) are combined here as they have likely similar preservation conditions. Note that cultural layers—the remains of human activities—are often found in the entrance area of caves (Hahn 1983). (ii) Open-air camps with temporary waterlogged preservation due to lake transgression, and (iii) open-air finds from dry conditions not associated with cultural layers (Fig. 1, Table 1, online resource 1).

Excavations were undertaken from the 1870s to the early 1990s according to contemporary standards, which likely included washing directly at the site. The majority of samples were not treated for storage; exceptions are the teeth from Monruz which were prepared with dimethylketone-based hardener during archaeozoological analysis (W. Müller, pers. comm.). All material was stored in museums and archaeological collections since then. The age of the finds ranges between c. 47 and c. 12 ky. Twenty-seven samples with DNA preservation were ^{14}C dated using accelerator mass spectrometry (AMS) at the Ion Beam Physics laboratory of ETH Zurich, Switzerland, and calibrated with CalPal online (Danzeglocke et al. 2012). Additionally, conventional and AMS ^{14}C dates were assembled from the literature, including

Fig. 1 Location of investigated sites along the Jura Mountain chain with equid remains in Switzerland. *Black dots* indicate sites with amplifiable mtDNA. The sites are numbered according to Table 1 and online resource 1

sites without DNA preservation. For Brügglihöhle, the age was determined by typology (Bandi et al. 1952/53) and for both Münchenstein-Steinbruch and Allschwil-Ziegelei, where cultural layers are absent, according to faunal or geological indicators (LeTensorer and Niffeler 1993, see online resource 1). Multiple typing of the same individual was avoided by choosing the same skeletal elements and/or samples from different layers or in the case of Kohlerhöhle excluded in retrospect by archaeozoological individualisation of teeth.

Sample preparation, DNA extraction and PCR amplification

The outer surface of teeth and bones was removed with sandpaper, and cubes of ~1 cm^3 cut out with a Dremel® tool. The cubes were ground with a mixer mill (Retsch MM2, Schieritz & Hauenstein, Allschwil, Switzerland). DNA extraction followed the User Developed Protocol: "Purification of total DNA from compact animal bone using the DNeasy® Blood & Tissue Kit" (Qiagen, Basel, Switzerland) for less than 100 mg. At least one mock control was performed per 20 samples. All extracts were ultra-purified with water (molecular biology grade, Eppendorf, Allschwil, Switzerland) using 30 kD filter units (Amicon/Millipore, Zug, Switzerland) to remove potential inhibitors. The final eluate was 200 μl.

One 75 bp target of the mt d-loop covering nucleotide positions 15,696–15,730 (except primers, Xu and Anarson 1994) was PCR amplified in 25 μl volumes with primers Ec5f (5′ACCCCATCCAAGTCAAATCA) and Eac1r (5′ GGCTTGGTGATTAAGCTCGT) containing 1.5 U AmpliTaq Gold, 1× GeneAmp 10× PCR Gold Buffer (150 mM Tris–HCl, 500 mM KCl, pH 8.0) and 2 mM $MgCl_2$ (all Applied Biosystems, Hombrechtikon, Switzerland); 0.4 mM dNTP Mix (Promega, Dübendorf, Switzerland); 0.2 μM of each primer; 20 μg/μl bovine serum albumin (BSA, Roche, Basel, Switzerland), and up to 5 μl template DNA on a Mastercycler ProS (Eppendorf, Allschwil, Switzerland). The cycling conditions were 12 min initial denaturation, followed by 50 cycles of denaturation at 95 °C for 40 s, annealing at 52 °C for 30 s, and extension at 72 °C for 30 s, with a final extension of 60 s at 72 °C. Non-template controls were performed alongside all amplifications. To overcome any potential PCR inhibition, DNA extracts were diluted 1:10 (Kemp et al. 2014). Firstly, 0.3 and 3 μl template DNA for each sample and extraction were PCR targeted. If not successful, the amplification was repeated with 0.5 and 5 μl

Table 1 Overview of archaeological sites (numbers correspond to Fig. 1), age bins, type of sites, years of excavation, storage time bins, tissue types, mtDNA d-loop amplification, and PCR performance of positive samples expressed in percentage of total PCR

Site		Date range of site (ky BP)	Age bin (ky BP)	Type of site	Year(s) of excavation	Storage time bin (y)	Samples with mt d-loop amplification (total samples)				PCR performance: Positive PCR (total PCR)	
							Teeth		Bones			
							n	%	*n*	%	*n*	%
1	Riehen-Ausserberg	47–45	47–37	Open, dry	1967	20–50	–	–	–(8)	0	–	–
2	Münchenstein-Steinbruch	Pre-LGM	47–37	Open, dry	1919 *et seq.*	70–90	–(4)	0	–	–	–	–
3	Allschwil-Ziegelei	Pre-LGM	47–37	Open, dry	1923	70–90	–(3)	0	–	–	–	–
4	Schalberghöhle	41–37	47–37	Cave	1926–1927	70–90	3 (6)[a]	67	–	–	10 (31)	32
		12.5	17–12				1				3 (10)	30
5	Kohlerhöhle	24–19	23	Cave	1934–38	70–90	11 (11)	100	–	–	36 (44)	82
		15–13	17–12				12 (15)	80	–	–	40 (90)	44
6	Kesslerloch	18–13	17–12	Cave	1874, 1883, 1898–1999, 1902–1903	110–140	29 (48)	60	14 (19)	74	75 (105)/ 28 (58)	71/ 48
7	Hauterive-Champréveyres	18–13	17–12	Open, temporally wetland	1983–1986	20–50	–(6)	0	–	–	–	–
8	Käsloch	17–15	17–12	Cave	1905	110–140	3 (4)	75	–	–	6 (22)	27
9	Monruz	17–15	17–12	Open, temporally wetland	1989–1992	20–50	–(10)	0	–(19)	0	–	–
10	Schweizersbild	17–13.5	17–12	Abri	1891–1893	110–140	6 (11)	55	–	–	19 (25)	76
11	Abri Neumühle	14.5–12	17–12	Abri	1965	20–50	1 (1)	100	–	–	3 (4)	75
12	Birseck-Ermitage	14	17–12	Cave	1910, 1914–1922	110–140	–(3)	0	–	–	–	–
13	Rislisberghöhle	16–12.5	17–12	Cave	1971, 1973	20–50	4 (13)	31	–	–	9 (29)	31
14	Brügglihöhle	Magdalenian?	17–12	Cave	1940, 1943, 1951–1952	70–90	–	–	–(1)	0	–	–
	Totals						70 (135)	52	14 (47)	30	229 (418)	55

For detailed locations, dates and references, see online resource 1

[a] Two samples without preserved mtDNA have not been ^{14}C dated

template DNA; in case of only negative results, the sample was deemed failed. Further targets were amplified for positive specimens (data not shown). All PCR products were cloned with the TOPO TA Cloning Kit (Invitrogen, Zug, Switzerland) following the manufacturer's protocol, except that the reaction volume was halved. Generally, two clones of each PCR product were Sanger sequenced by Microsynth (Balgach, Switzerland), but the number varied from one to eight.

Data analysis

The potential effects of study sites (cave/abri, open dry and wetland sites), tissue type (tooth or bone), storage time, and sample age on PCR amplification and performance and the amount of post mortem damage were analysed with linear models using R (R Development Core Team 2014). Due to a clustering of excavations in certain years, storage time was assigned to three categories: 20–50 years (n=57), 70–90 years (n=40), and 110–140 years (n=85). Accordingly, age bins were defined: 17–12 ky (n=151), 23 ky (n=11), and 47–37 ky (n=20). Data were described with bar and boxplot charts. First, general amplification success, i.e. whether mtDNA was amplifiable from a sample or not, was evaluated with the aforementioned factors and with binomial distributed errors using a generalised linear model (GLM) and a χ^2 test. From then on, only positive samples were considered. Second, PCR performance per individual sample was recorded by concatenating the number of positive and negative PCR attempts and evaluated as mentioned but with quasibinomial distributed errors. Third, for evaluation of miscoding lesions, sequences were edited and aligned by eye with BioEdit (Hall 1999), and base modifications that could not be reproduced were considered inconsistent. We determined the number of miscoding lesions per sample and position as follows: identical clones from the same amplification were counted as one

sequence, and in case further clones from the same amplification differed, they were counted separately each. Because the estimation of the *true* rate of damage is virtually impossible with a conventional PCR approach, C→T substitutions were used as a *proxy* for DNA damage (Brotherton et al. 2007; Vives et al. 2008). For standardisation, the counted misincorporated thymines were divided by the total number of cytosines for each specimen. To examine the influence of the mentioned factors on the amount of damage in individual specimens, we applied the GLM model with inverse Gaussian distributed errors.

Precautions and authenticity

Established standards in aDNA research at the Integrative Prehistory and Archaeological Science (IPAS) were adhered to (e.g. Schlumbaum et al. 2010). In detail: All ancient DNA work (pre-PCR) was performed in dedicated, physically separated laboratories for sample preparation, DNA extraction and PCR setup in a different building than the post-PCR laboratory following a strict one-way policy. Experiments were performed freshly showered and wearing dedicated freshly washed clothes. Gloves and sleeves were changed regularly. Bones and teeth were cut in an acrylic glass box equipped with an UV lamp and a vacuum cleaner to remove bone dust. After each working step, surfaces and tools were cleaned with soap and commercial bleach (Javel-Wasser, Migros, Zurich, Switzerland) and UV irradiated for at least 30 min. Diamond cutting disks were cleaned with soap and ethanol followed by 30 min UV irradiation from each side. Mixer mill beakers were cleaned with soap, quartz sand and 30 min bleach incubation. PCR was set up in a laminar flow cabinet equipped with an UV lamp. Plastic ware was UV irradiated prior to use. We did not perform sample preparation, extraction and PCR on the same day. No modern horse DNA was analysed in the laboratories, and none of the coworkers had contact with living horses. At the same time, archaeological samples of other species were processed in the aDNA laboratories, but cross-contamination was never documented. PCR products in the negative controls were always either microorganisms or unidentifiable according to GenBank Blast search. Each target was validated with at least two independent extractions and three PCR products; all products were cloned and Sanger sequenced.

Results

PCR amplification success

We investigated equid remains from 14 Palaeolithic sites comprising caves, rock shelters (abri deposits) and open dry and wetland sites in Switzerland (Fig. 1, Table 1, online resource 1). Eighty four out of 182 remains (46 %) from seven sites have yielded equine mtDNA. DNA amplification of the 75 bp target was strongly related to depositional condition; all positive samples stem from cave and abri deposits (Fig. 2a, Table 2). This results in a success rate for cave/abri sites of 64 % (84/132 remains). Amplification success varied between the cave/abri sites; 23/26 samples (88 %) from Kohlerhöhle but only 4/13 samples (31 %) from Rislisberghöhle and none from both Brügglihöhle (n=3) and Birseck-Ermitage (n=1) had amplifiable mtDNA preserved (online resource 2). No mtDNA was obtained from any of the tested samples from five Palaeolithic open dry and temporally wetland sites. A higher percentage of teeth than bones had amplifiable mtDNA preserved; however, the difference is not significant (Fig. 2b). Both storage time after excavation and age had a significant relation to DNA survival, albeit not in a linear way (Fig. 2c, d). For example, the sites excavated most recently include all temporally wetland sites, considerably lowering the success rate within the 20–50 years bin. Moreover, the temporally waterlogged samples in the 17–12 ky age bin and the dry open sites in the 47–37 ky age bin obviously complicate the interpretation of the results.

PCR performance

For a detailed analysis of those samples that yielded mtDNA, we tested whether the factors tissue type, storage time and sample age influenced PCR performance. We did not detect differences in the amplification performance between teeth and bones (Fig. 3a, Table 3). Storage time had an inverse relationship to PCR performance: Specimens that had been unearthed before or around 1900 performed better than those excavated later (Fig. 3b). Sample age has a significant influence on PCR performance (Fig. 3c, see also online resource 2B). As expected, the oldest specimens amplified generally worse, but note that the 47–37 ky bin is only represented by three positive samples from Schalberghöhle. The performance differences between the individual cave sites were more pronounced than the differences between the age bins.

Miscoding lesions

We evaluated a total of 7,980 nucleotides from 228 sequences (see "Methods") and identified 62 C→T substitutions (Table 4) resulting in a deamination rate of 2 % for cytosines. In agreement with a previous study (Vives et al. 2008), we found only eight of G→A substitutions (Table 4), suggesting preferential amplification of the light strand. Based solely on C→T changes, none of the factors influenced the pattern of misincorporations. We observed large variation among individuals. More than half of the samples (47/84) exhibited no damage at all; 36 specimens were deaminated between 1 and

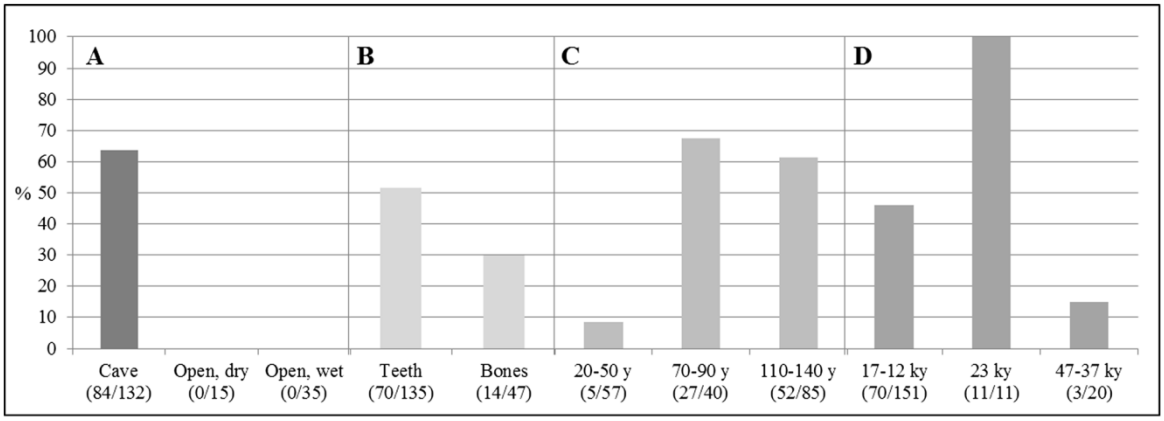


Fig. 2 Percentage of Palaeolithic equid samples with 75 bp target of amplifiable mtDNA in Switzerland. **a** In relation to original site (cave/abri, open and dry, open and wet); **b** in relation to tissue type (tooth, bone); **c** in relation to storage time after excavation (20–50 years, 70–90 years, 110–140 years); and **d** in relation to age (17–12 ky, 23 ky, 47–37 ky)

7.5 %, and two had damage rates as high as 13 and 15 %, respectively. The three oldest samples show on average more deaminations, but the difference is not significant (Fig. 4a–c, Table 5).

Intra-site comparison

We expected differences in mtDNA preservation, individual PCR amplification success and post mortem damage patterns to be linked with finds buried in different positions within the cave, with tissue type and age. The caves Kesslerloch and Kohlerhöhle allow investigating any potential effect of these parameters in the absence of among-site variation (online resource 2). Kesslerloch is the only cave where both equid bones and teeth were unearthed and genetically analysed; all samples are in the same age bin. Kohlerhöhle specimens can be assigned to two age bins: 23 ky and 17–12 ky; these are all teeth. For both sites, samples were deposited in different parts of the cave. Fourteen samples from Kesslerloch were excavated near the entrance (area s) and 27 more than 6 m distance from the two openings (area m–n and c; see online resource 3; Merk 1876; Nüesch et al. 1904; Heierli 1907). DNA preservation, PCR performance and damage rate were not different throughout the cave. Whereas mtDNA was preserved in a higher percentage of bones than teeth, in contrast to the overall observations in the entire data set, the performance of the 29 positive teeth was significantly better than of the 14 positive bones. Both tissues showed similar damage patterns (online resource 4).

The cave Kohlerhöhle is almost 15 m long with an additional 3 m abri area (online resource 5; Lüdin 1938). The 23 ky old specimens (n=11) were all unearthed from the rear 6 m of the cave, some sticking within the maxilla (foramina apicale not protected); mtDNA was preserved in all teeth. In contrast, the 15 younger samples (15 to 13 ky old) were dispersed all over the cave and abri area, but albeit three of the teeth had no mtDNA preserved (one from the hall, one from the abri and one of unknown deposit), we also did not detect differences in preservation, PCR performance and deamination rate between interior and entrance areas. Comparison of the age bins confirmed the observation that the 23 ky specimens performed better than the younger samples in the same cave (online resource 6).

Table 2 Effect of study sites (cave/abri, open and dry, open and wet); tissue type (tooth, bone); storage time after excavation (20–50 years, 70–90 years, 110–140 years); and sample age (17–12 ky, 23 ky, 47–37 ky) on mtDNA preservation (PCR success) of Palaeolithic equid remains from Switzerland

	Df	χ^2	p
Site	2	79.414	<0.0001***
Tissue type	1	0.422	0.52
Storage time	2	10.213	0.006**
Sample age	2	6.056	0.05*

Significance codes: 0.001=***, 0.01=**, 0.05=*

Discussion

Long-term DNA survival in archaeological contexts is complex and influenced by a number of parameters, mainly probably by temperature (Smith et al. 2003; Lamers et al. 2009; Allentoft et al. 2012; Sawyer et al. 2012). The present data set, though comprising all known Palaeolithic sites in Switzerland with more than one horse remain, is heterogeneous: All open dry sites predate the European LGM, waterlogged open sites are both spatially and temporally close to each other, and the amount of pre-Magdalenian specimens from caves is low.

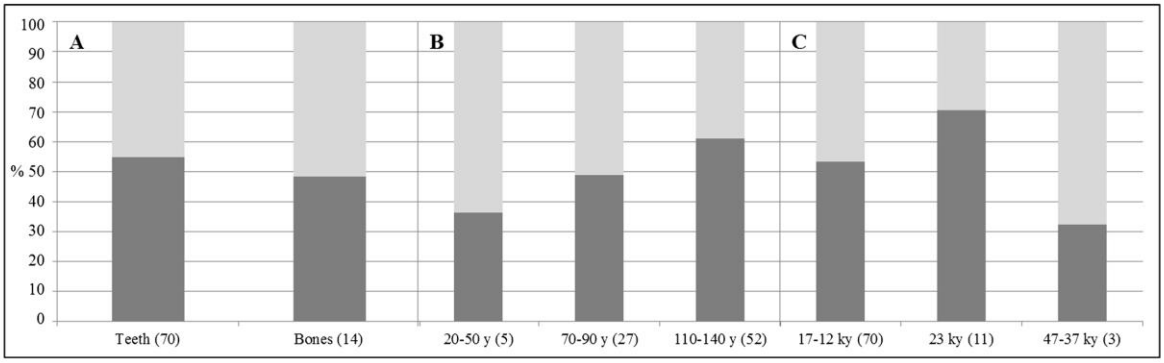


Fig. 3 Proportion of positive (*dark grey*) to negative (*light grey*) PCR products obtained from positive Palaeolithic equid samples from cave and abri sites in Switzerland (PCR performance). **a** Effect of tissue type (tooth, bone); **b** effect of age (17–12 ky, 23 ky, 47–37 ky); and **c** effect of storage time after excavation (20–50 years, 70–90 years, 110–140 years)

Teeth were overrepresented in our study, mirroring the archaeological record concerning horses from the Aurignacian to the Magdalenian in the region Swabian/Swiss Jura (Niven 2003; Napierala 2008). Moreover, the often scarce documentation and ambiguous definition of depositional contexts in published data makes it difficult to compare our results. However, in archaeology, and in particular archaeogenetics, representativeness is hardly expected. Despite the heterogeneity of the data set, our results support the finding that within the time frame discussed here, survival of mtDNA is mainly determined by in situ burial conditions. We demonstrate for the first time that Palaeolithic cave depositions are a much better source of ancient DNA than open dry and wetland sites from the same time period, even when morphological and in some cases also collagen preservation is good. The majority of caves and abris provided preservation conditions good enough to allow PCR amplification of mitochondrial DNA in more than 50 % of specimens. One reason for the superior DNA preservation may be the fact that remains at the rock shelters as well as at the cave entrance areas were rapidly sedimented with chalky soil protecting against hydrolytic and oxidative damage. Inside the caves, cultural layers were also covered by sinter (Bally 1908; Sedlmeier 1993; Napierala 2008), a calcareous deposit that develops when water dilutes calcium carbonate from limestone. These speleothems can form thick layers and seem to protect organic residues largely from microbes. In general, DNA preservation in the caves investigated was comparable to studies on large as well as small mammals from other European cave sites (e.g. Edwards et al. 2010; Stiller et al. 2010; Münzel et al. 2011; Pruvost et al. 2011; Brace et al. 2012). Two caves did not preserve equid mtDNA. This result might be explained by the destruction of cultural layers at Birseck-Ermitage to make the cave publicly accessible in the nineteenth century (Sarasin et al. 1918). Similarly, the roof of Brügglihöhle had collapsed, possibly amid the 1356 earthquake (Lambert et al. 2005), exposing the archaeological layer (Bandi et al. 1952/53); these factors probably caused the general poor preservation status of the faunal remains.

The open dry sites are comparatively old based on ^{14}C dated samples from Riehen Ausserberg (>45 ky BP) and additional geological and faunal indicators (LeTensorer and Niffeler 1993), but equine mtDNA of similar age has been obtained from a cave in the Swabian Jura (Germany, Weinstock et al. 2005). Thus, it seems plausible that the dynamics of the soil itself (root penetration, pedogenesis, eluviation), soil-dwelling microorganisms as well as seasonal temperature and humidity fluctuation have promoted DNA decay in open sites. The Magdalenian remains at Champréveyres and Monruz were temporarily waterlogged due to transgression of Lake Neuchâtel (Coope and Elias 2000) which might explain the failed amplifications because

Table 3 Effect of tissue type (tooth, bone), storage time after excavation (20–50 years, 70–90 years, 110–140 years); and sample age (17–12 ky, 23 ky, 47–37 ky) on PCR performance of Palaeolithic equid samples from cave/abri sites in Switzerland

	Df	χ^2	*p*
Tissue type	1	0.847	0.44
Storage	2	14.651	0.006**
Sample age	2	14.778	0.005**

Significance codes: 0.001=***, 0.01=**, 0.05=*

Table 4 Number (n) of adenines (A), cytosines (C), guanines (G), and thymines (T) in all sequences and miscoding lesions (transitions) observed in a 75 bp mtDNA d-loop fragment from Palaeolithic equid samples from cave/abri sites in Switzerland

	n	Transitions
A	2,273	4
C	2,969	62
G	691	8
T	2,047	3

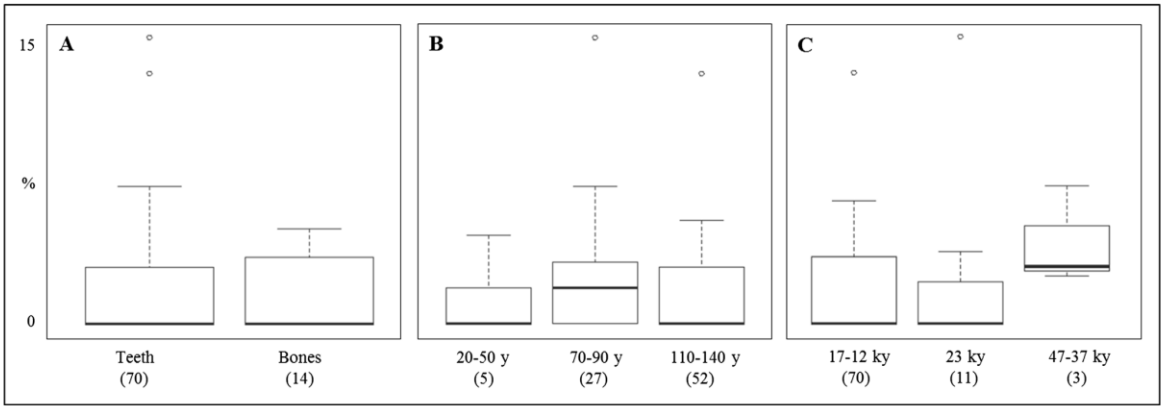


Fig. 4 mtDNA damage as represented by percentage of C→T substitutions obtained from positive Palaeolithic equid samples from cave and abri sites in Switzerland. **a** Effect of tissue type (tooth, bone); **b** effect of storage time after excavation (20–50 years, 70–90 years, 110–140 years); and **c** effect of age (17–12 ky, 23 ky, 47–37 ky)

the alternation between wet and dry environment is not beneficial for preservation in general (Reiche et al. 2003; Schlumbaum and Edwards 2013). In this context particularly, PCR inhibition can be a serious problem for the amplification of ancient DNA (Kemp et al. 2014). We took a range of measures to overcome potential inhibitors (ultra-purification and dilution of extracts, adding BSA to the PCR), but we cannot rule out that it affected our overall results.

It is commonly assumed that teeth are preferable for ancient DNA analyses. Their composition makes them less prone to contamination, protects DNA against deamination and leads to better preservation (Lindahl 1993; Gilbert et al. 2005; Adler et al. 2011; Campos et al. 2012), notably if articulated to the jaw bones (Higgins and Austin 2013). In the complete data set, we did not detect significant preservation and performance differences between teeth and bones; note that all teeth had to be considered loose because their roots were exposed. However, bones were only amplifiable from one cave site (Kesslerloch) and are generally underrepresented in the archaeological record. Thus, any differences in preservation between skeletal elements (bone vs teeth) may have been masked by this imbalance. Previous publications have been contradictory on this topic (e.g. Götherström et al. 2002; Ricaut et al. 2005; Miloš et al. 2007; Pruvost et al. 2008; Higgins and Austin 2013).

Table 5 Effect of tissue type (tooth, bone), storage time after excavation (20–50 years, 70–90 years, 110–140 years); and sample age (17–12 ky, 23 ky, 47–37 ky) on mtDNA damage as represented by C→T substitutions in Palaeolithic equid samples from cave and abri sites in Switzerland

	Df	F	*p*
Tissue type	1	5.8527e-06	0.3808
Storage	2	7.6108e-06	0.6054
Sample age	2	1.3813e-05	0.4041

Significance codes: 0.001=***, 0.01=**, 0.05=*

The samples we analysed have been stored in archaeological collections up to 140 years. Pruvost et al. (2007) have pointed out that freshly excavated bones would be the first choice for ancient DNA analyses, but in fact, most archaeogenetic studies rely on museum specimens. Similar to Gravlund et al. (2012), we can confirm the hypothesis that many stored samples still have genetic information preserved although they might not have been treated with the precautions that are recommended today for aDNA studies (e.g. Bollongino et al. 2008; Pruvost et al. 2008; Matisoo-Smith and Horsburgh 2012).

The observation that specimens from the 23 ky age bin were better preserved that those from the 17–12 ky age bin even when compared within the same site (Kohlerhöhle) might reflect the influence of temperature at the time of deposition (Smith et al. 2003) which was considerably lower during the LGM (Buoncristiani and Campy 2011; Cupillard et al. 2014).

We did not observe a significant increase in the amount of C→T substitutions in older samples in contrast to the observation from NGS data (Sawyer et al. 2012), probably because PCR does not allow capturing the ends of DNA template strands, where the majority of deamination events occur (Briggs et al. 2007; Brotherton et al. 2007).

In contrast to genomic studies that investigate single samples and can afford specially tailored extraction and sequencing methods (e.g. Meyer et al. 2012; Orlando et al. 2013; Meyer et al. 2014; Olalde et al. 2014), many archaeological studies require routine access to aDNA from a larger number of samples (e.g. Edwards et al. 2007; Stiller et al. 2010; Münzel et al. 2011; Brace et al. 2012). On this note, we scored successful PCR amplification and PCR performance in positive samples as key variables rather than applying more sophisticated measures of DNA preservation such as quantitative PCR. In many archaeological studies aiming to apply

aDNA analyses, assessing the chances for a successful amplification using traditional PCR is the first important parameter to justify the damage inflicted on the sample as well as the amount of working time and money invested.

Conclusion

By evaluating mtDNA d-loop amplification, performance and damage patterns in 182 Palaeolithic horse remains up to 47 ky in age from different depositional contexts in Switzerland, we found that the type of depositional environment is the most important factor affecting DNA preservation. Cave sites provide more favourable environments for DNA preservation than open sites. Moreover, both equid teeth and bones are suitable sources of mtDNA. In our data set, the factors storage time after excavation and sample age were of influence but not in a linear way. In the future, a systematic evaluation of younger samples with different depositional contexts and excavation biographies should reveal the eligibility for ancient DNA analyses of further site types, e.g. the famous Neolithic lacustrine sites, or Celtic and Roman food waste for genetic analyses. Finally, by saying that Palaeolithic faunal remains from caves offer better chances to DNA preservation in temperate areas, the fast development in technology may come up with solutions for samples from other burial conditions in the future, in particular for specimen of interest not preserved in caves.

Acknowledgments Samples were kindly provided by Archäologische Bodenforschung Basel-Stadt (Riehen-Ausserberg), Naturhistorisches Museum Basel (Münchenstein-Steinbruch, Allschwil-Ziegelei, Schalberghöhle, Birseck-Ermitage), Archäologie und Kantonsmuseum Basel-Landschaft (Brügglihöhle, Kohlerhöhle), Archäologischer Dienst Bern (Abri Neumühle), Université de Neuchâtel (Hauterive-Champréveyres, Monruz), Kantonsarchäologie Schaffhausen (Kesslerloch, Schweizersbild), Kantonsarchäologie Solothurn (Rislisberg, Käsloch) and by J. Sedlmeier. The authors thank R. Jagher, W. Müller, H. P. Rusterholz, as well as M. Collins and two anonymous reviewers for comments on the manuscript. This study was funded by Swiss National Science Foundation (K-31K1_120528/1), Freiwillige Akademische Gesellschaft Basel, Nikolaus und Bertha Burckhardt-Bürgin-Foundation.

References

Adler CJ, Haak W, Donlon D, Cooper A (2011) Survival and recovery of DNA from ancient teeth and bones. J Archaeol Sci 38(5):956–964. doi:10.1016/j.jas.2010.11.010

Allentoft ME, Collins M, Harker D, Haile J, Oskam CL, Hale ML, Campos PF, Samaniego JA, Gilbert MTP, Willerslev E, Zhang G, Scofield RP, Holdaway RN, Bunce M (2012) The half-life of DNA in bone: measuring decay kinetics in 158 dated fossils. Proc R Soc B 279(1748):4724–4733. doi:10.1098/rspb.2012.1745

Anderson CNK, Ramakrishan U, Chan YL, Hadly EA (2005) Serial SimCoal: a population genetics model for data from multiple populations and points in time. Bioinformatics 21(8):1733–1734

Bally E (1908) Höhlenfunde im sog. Käsloch (Kt. Solothurn). Anz Schweiz Altertumskunde 10:1–12

Bandi HG, Lüdin C, Mamber W, Schaub S, Schmid E, Welten M (1952/53) Die Brügglihöhle an der Kohlholzhalde bei Nenzlingen (Kt. Bern), eine neue Fundstelle des Spätmagdalénien im untern Birstal. Jahrb Bernschen Historischen Mus 32/33:45–76

Bollongino R, Tresset A, Vigne JD (2008) Environment and excavation: pre-lab impacts on ancient DNA analyses. C R Palevol 7(2/3):91–98. doi:10.1016/j.crpv.2008.02.002

Brace S, Palkopoulou E, Dalén L, Lister AM, Miller R, Otte M, Germonpré M, Blockley SPE, Stewart JR, Barnes I (2012) Serial population extinctions in a small mammal indicate Late Pleistocene ecosystem instability. PNAS. doi:10.1073/pnas.1213322109

Briggs AW, Stenzel U, Johnson PLF, Green RE, Kelso J, Prüfer K, Meyer M, Krause J, Ronan MT, Lachmann M, Pääbo S (2007) Patterns of damage in genomic DNA sequences from a Neandertal. Proc R Soc B 104(37):14616–14621. doi:10.1073/pnas.0704665104

Brotherton P, Endicott P, Sanchez JJ, Beaumont M, Barnett R, Austin J, Cooper A (2007) Novel high-resolution characterization of ancient DNA reveals C>U-type base modification events as the sole cause of post mortem miscoding lesions. Nucleic Acids Res 35(17):5717–5728. doi:10.1093/nar/gkm588

Buoncristiani JF, Campy M (2011) Quaternary glaciations in the French Alps and Jura. In: Ehlers J, Gibbard PL, Hughes PD (eds) Quaternary glaciations – extent and chronology. Elsevier, Amsterdam, pp 117–126

Campos PF, Kristensen T, Orlando L, Sher A, Kholodova MV, Gotherström A, Hofreiter M, Drucker DG, Kosintsev P, Tikhonov A, Baryshnikov GF, Willerslev E, Gilbert MTP (2010) Ancient DNA sequences point to a large loss of mitochondrial genetic diversity in the saiga antelope (Saiga tatarica) since the Pleistocene. Mol Ecol 19(22):4863–4875. doi:10.1111/j.1365-294X.2010.04826.x

Campos PF, Craig OE, Turner-Walker G, Peacock E, Willerslev E, Gilbert MTP (2012) DNA in ancient bone—where is it located and how should we extract it? Ann Anat 194(1):7–16. doi:10.1016/j.aanat.2011.07.003

Collins MJ, Penkman KEH, Rohland N, Shapiro B, Dobberstein RC, Ritz-Timme S, Hofreiter M (2009) Is amino acid racemization a useful tool for screening for ancient DNA in bone? Proc R Soc B 276(1669):2971–2977. doi:10.1098/rspb.2009.0563

Coope GR, Elias SA (2000) The environment of Upper Palaeolithic (Magdalenian and Azilian) hunters at Hauterive-Champréveyres, Neuchâtel, Switzerland, interpreted from coleopteran remains. J Quat Sci 15(2):157–175. doi:10.1002/(sici)1099-1417(200002)15:2<157::aid-jqs478>3.0.co;2-k

Cupillard C, Magny M, Bocherens H, Bridault A, Bégeot C, Bichet V, Bossuet G, Drucker DG, Gauthier E, Jouannic G, Millet L, Richard H, Rius D, Ruffaldi P, Walter-Simmonet AV (2014) Changes in ecosystems, climate and societies in the Jura Mountains between 40 and 8 ka cal BP. Quat Int. doi:10.1016/j.quaint.2014.05.032

Dabney J, Meyer M, Pääbo S (2013) Ancient DNA damage. Cold Spring Harb Perspect Biol. doi:10.1101/cshperspect.a012567

Danzeglocke U, Jöris O, Weniger B (2012) CalPal-2007 online. Accessed Dec. 2012-march 2013

Deagle B, Eveson J, Jarman S (2006) Quantification of damage in DNA recovered from highly degraded samples—a case study on DNA in faeces. Front Zool 3(1):1–10. doi:10.1186/1742-9994-3-11

Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol Biol Evol 29(8):1969–1973. doi:10.1093/molbev/mss075

Edwards CJ, Bollongino R, Scheu A, Chamberlain A, Tresset A, Vigne JD, Baird JF, Larson G, Ho SYW, Heupink TH, Shapiro B, Freeman AR, Thomas MG, Arbogast RM, Arndt B, Bartosiewicz L, Benecke N, Budja M, Chaix L, Choyke AM, Coqueugniot E, Döhle HJ, Göldner H, Hartz S, Helmer D, Herzig B, Hongo H, Mashkour M, Özdogan M, Pucher E, Roth G, Schade-Lindig S, Schmölcke U, Schulting RJ, Stepahn E, Uerpmann HP, Vörös I, Voytek B, Bradley D, Burger J (2007) Mitochondrial DNA analysis shows a Near Eastern Neolithic origin for domestic cattle and no indication of domestication of European aurochs. Proc R Soc B 274:1377–1385. doi:10.1098/rspb.2007.0020

Edwards CJ, Magee DA, Park SDE, McGettigan PA, Lohan AJ, Murphy A, Finlay EK, Shapiro B, Chamberlain AT, Richards MB, Bradley DG, Loftus BJ, MacHugh DE (2010) A complete mitochondrial genome sequence from a Mesolithic wild aurochs (Bos primigenius). PLoS One 5(2):e9255. doi:10.1371/journal.pone.0009255

Excoffier L, Lischer HE (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10(3):564–567

Geigl EM (2002) On the circumstances surrounding the preservation and analysis of very old DNA. Archaeometry 44:337–342

Gilbert MTP, Bandelt H-J, Hofreiter M, Barnes I (2005) Assessing ancient DNA studies. Trends Ecol Evol 20(10):541–544. doi:10.1016/j.tree.2005.07.005

Gilbert MTP, Janaway RC, Tobin DJ, Cooper A, Wilson AS (2006) Histological correlates of post mortem mitochondrial DNA damage in degraded hair. Forensic Sci Int 156(2/3):201–207. doi:10.1016/j.forsciint.2005.02.019

Gilbert MTP, Binladen J, Miller W, Wiuf C, Willerslev E, Poinar H, Carlson JE, Leebens-Mack JH, Schuster SC (2007) Recharacterization of ancient DNA miscoding lesions: insights in the era of sequencing-by-synthesis. Nucleic Acids Res 35(1):1–10. doi:10.1093/nar/gkl483

Götherström A, Collins MJ, Angerbjörn A, Lidén K (2002) Bone preservation and DNA amplification. Archaeometry 44(3):395–404. doi:10.1111/1475-4754.00072

Gravlund P, Aaris-Sørensen K, Hofreiter M, Meyer M, Bollback JP, Noe-Nygaard N (2012) Ancient DNA extracted from Danish aurochs (Bos primigenius): genetic diversity and preservation. Ann Anat 194(1):103–111. doi:10.1016/j.aanat.2011.10.011

Guarino FM, Angelini F, Odierna G, Bianco MR, Di Bernardo G, Forte A, Cascino A, Cipollaro M (2000) Detection of DNA in ancient bones using histochemical methods. Biotech Histochem 75(2):110–117

Hahn J (1983) Eiszeitliche Jäger zwischen 35000 und 15000 vor heute. In: Müller-Beck H (ed) Urgeschichte in Baden-Württemberg. Konrad Theiss, Stuttgart, pp 273–330

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Hansen AJ, Mitchell DL, Wiuf C, Paniker L, Brand TB, Binladen J, Gilichinsky DA, Rønn R, Willerslev E (2006) Crosslinks rather than strand breaks determine access to ancient DNA sequences from frozen sediments. Genetics 173(2):1175–1179. doi:10.1534/genetics.106.057349

Haynes S, Searle JB, Bretman A, Dobney KM (2002) Bone preservation and ancient DNA: the application of screening methods for predicting DNA survival. J Archaeol Sci 29(6):585–592. doi:10.1006/jasc.2001.0731

Heierli J (1907) Das Kesslerloch bei Thaingen. Neue Denkschriften Schweizerischen Naturforschenden Ges 43

Heyn P, Stenzel U, Briggs AW, Kircher M, Hofreiter M, Meyer M (2010) Road blocks on paleogenomes—polymerase extension profiling reveals the frequency of blocking lesions in ancient DNA. Nucleic Acids Res 38(16):e161. doi:10.1093/nar/gkq572

Higgins D, Austin JJ (2013) Teeth as a source of DNA for forensic identification of human remains: a review. Sci Justice 53:433–441. doi:10.1016/j.scijus.2013.06.001

Hofreiter M, Serre D, Poinar HN, Kuch M, Paabo S (2001) Ancient DNA. Nat Rev Genet 2(5):353–359. doi:10.1038/35072071

Hoke N, Burger J, Weber C, Benecke N, Grupe G, Harbeck M (2011) Estimating the chance of success of archaeometric analyses of bone: UV-induced bone fluorescence compared to histological screening. Palaeogeogr Palaeoclimatol Palaeoecol 310(1/2):23–31. doi:10.1016/j.palaeo.2011.03.021

Höss M (1995) Ancient DNA. Horm Res 43:118–120

Hughes PD, Gibbard PL, Ehlers J (2013) Timing of glaciation during the last glacial cycle: evaluating the concept of a global 'Last Glacial Maximum' (LGM). Earth Sci Rev 125:171–198. doi:10.1016/j.earscirev.2013.07.003

Kemp BM, Monroe C, Judd KG, Reams E, Grier C (2014) Evaluation of methods that subdue the effects of polymerase chain reaction inhibitors in the study of ancient and degraded DNA. J Archaeol Sci 42:373–380. doi:10.1016/j.jas.2013.11.023

Lambert J, Winter T, Dewez TJB, Sabourault P (2005) New hypotheses on the maximum damage area of the 1356 Basel earthquake (Switzerland). Quat Sci Rev 24(3/4):381–399. doi:10.1016/j.quascirev.2004.02.019

Lamers R, Hayter S, Matheson C (2009) Postmortem miscoding lesions in sequence analysis of human ancient mitochondrial DNA. J Mol Evol 68(1):40–55. doi:10.1007/s00239-008-9184-3

LeTensorer J-M, Niffeler U (eds) (1993) SPM I Paläolithikum und Mesolithikum. Die Schweiz vom Paläolithikum bis zum frühen Mittelalter. Schweizerische Gesellschaft für Ur- und Frühgeschichte, Basel

Lindahl T (1993) Instability and decay of the primary structure of DNA. Nature 362:709–715

Lorenzen ED, Nogues-Bravo D, Orlando L, Weinstock J, Binladen J, Marske KA, Ugan A, Borregaard MK, Gilbert MTP, Nielsen R, Ho SYW, Goebel T, Graf KE, Byers D, Stenderup JT, Rasmussen M, Campos PF, Leonard JA, Koepfli K-P, Froese D, Zazula G, Stafford TW, Aaris-Sorensen K, Batra P, Haywood AM, Singarayer JS, Valdes PJ, Boeskorov G, Burns JA, Davydov SP, Haile J, Jenkins DL, Kosintsev P, Kuznetsova T, Lai X, Martin LD, McDonald HG, Mol D, Meldgaard M, Munch K, Stephan E, Sablin M, Sommer RS, Sipko T, Scott E, Suchard MA, Tikhonov A, Willerslev R, Wayne RK, Cooper A, Hofreiter M, Sher A, Shapiro B, Rahbek C, Willerslev E (2011) Species-specific responses of Late Quaternary megafauna to climate and humans. Nature 479(7373):359–364. doi:10.1038/nature10574

Lüdin C (1938) Kohlerhöhle bei Brislach (Amt Laufen, Bern). Jahrb Schweizerischen Ges für Ur- und Frühgeschichte 30:61–64

Matisoo-Smith E, Horsburgh KA (2012) DNA for archaeologists. Left Coast Press, Walnut Creek

Merk C (1876) Excavations at the Kesslerloch near Thayngen, Switzerland: a cave of the reindeer period. Longman, Green and Co., London

Meyer M, Kircher M, Gansauge MT, Li H, Racimo F, Mallick S, Schraiber JG, Jay F, Prüfer K, de Filippo C, Sudmant PH, Alkan C, Fu Q, Do R, Rohland N, Tandon A, Siebauer M, Green RE, Bryc K, Briggs AW, Stenzel U, Dabney J, Shendure J, Kitzman J, Hammer MF, Shunkov MV, Derevianko AP, Patterson N, Andrés AM, Eichler EE, Slatkin M, Reich D, Kelso J, Pääbo S (2012) A high-coverage genome sequence from an archaic Denisovan individual. Science 338:222–226. doi:10.1126/science.1224344

Meyer M, Fu Q, Aximu-Petri A, Glocke I, Nickel B, Arsuaga JL, Martínez I, Gracia A, de Bermúdez Castro JM, Carbonell E, Pääbo S (2014) A mitochondrial genome sequence of a hominin from Sima de los Huesos. Nature 505:403–406. doi:10.1038/nature12788

Miloš A, Selmanović A, Smajlović L, Huel RLM, Katzmarzyk C, Rizvić A, Parsons TJ (2007) Success rates of nuclear short tandem repeat typing from different skeletal elements. Croat Med J 48(4):486–493

Mitchell D, Willerslev E, Hansen A (2005) Damage and repair of ancient DNA. Mutat Res 571(1/2):265–276. doi:10.1016/j.mrfmmm.2004.06.060

Molak M, Ho SYW (2011) Evaluating the impact of post-mortem damage in ancient DNA: a theoretical approach. J Mol Evol 73(3/4):244–255. doi:10.1007/s00239-011-9474-z

Münzel SC, Stiller M, Hofreiter M, Mittnik A, Conard NJ, Bocherens H (2011) Pleistocene bears in the Swabian Jura (Germany): genetic replacement, ecological displacement, extinctions and survival. Quatern Int 245(2):225–237. doi:10.1016/j.quaint.2011.03.060

Napierala H (2008) Die Tierknochen aus dem Kesslerloch. Neubearbeitung der paläolithischen Fauna, vol 2. Beiträge zur Schaffhauser Archäologie. Baudepartement des Kantons Schaffhausen, Kantonsarchäologie, Schaffhausen

Niven L (2003) Patterns of subsistence and settlement during the Aurignacian of the Swabian Jura, Germany. In: Zilhao J, d'Errico F (eds) The chronology of the Aurignacian and of the transitional technocomplexes: dating, stratigraphies, cultural implications. Lisboa, pp 199–212

Nüesch J, Studer T, Schötensack O (1904) Das Kesslerloch, eine Höhle aus paläolithischer Zeit: Neue Grabungen und Funde. Neue Denkschriften Schweizerischen Naturforschenden Ges 39(2)

Olalde I, Allentoft ME, Sánchez-Quinto F, Santpere G, Chiang CWK, DeGiorgio M, Prado-Martinez J, Rodríguez JA, Rasmussen S, Quilez J, Ramírez O, Marigorta UM, Fernández-Callejo M, Prada ME, Encinas JMV, Nielsen R, Netea MG, Novembre J, Sturm RA, Sabeti P, Marquès-Bonet T, Navarro A, Willerslev E, Lalueza-Fox C (2014) Derived immune and ancestral pigmentation alleles in a 7,000-year-old Mesolithic European. Nature 507(7491):225–228. doi:10.1038/nature12960

Orlando L, Ginolhac A, Zhang G, Froese D, Albrechtsen A, Stiller M, Schubert M, Cappellini E, Petersen B, Moltke I, Johnson PLF, Fumagalli M, Vilstrup JT, Raghavan M, Korneliussen T, Malaspinas A-S, Vogt J, Szklarczyk D, Kelstrup CD, Vinther J, Dolocan A, Stenderup J, Velazquez AMV, Cahill J, Rasmussen M, Wang X, Min J, Zazula GD, Seguin-Orlando A, Mortensen C, Magnussen K, Thompson JF, Weinstock J, Gregersen K, Roed KH, Eisenmann V, Rubin CJ, Miller DC, Antczak DF, Bertelsen MF, Brunak S, Al-Rasheid KAS, Ryder O, Andersson L, Mundy J, Krogh A, Gilbert MTP, Kjaer K, Sicheritz-Ponten T, Jensen LJ, Olsen JV, Hofreiter M, Nielsen R, Shapiro B, Wang J, Willerslev E (2013) Recalibrating Equus evolution using the genome sequence of an early Middle Pleistocene horse. Nature 499(7456):74–78. doi:10.1038/nature12323

Overballe-Petersen S, Orlando L, Willerslev E (2012) Next-generation sequencing offers new insights into DNA degradation. Trends Biotechnol 30(7):364–368. doi:10.1016/j.tibtech.2012.03.007

Pääbo S (1989) Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification. PNAS 86:1939–1943

Pääbo S, Poinar HN, Serre D, Jaenicke-Després V, Hebler J, Rohland N, Kuch M, Krause J, Vigilant L, Hofreiter M (2004) Genetic analyses from ancient DNA. Annu Rev Genet 38:645–679

Poinar HN, Höss M, Bada JL, Pääbo S (1996) Amino acid racemization and the preservation of ancient DNA. Science 272:864–866

Pruvost M, Schwarz R, Correia VB, Champlot S, Braguier S, Morel N, Fernandez-Jalvo Y, Grange T, Geigl E-M (2007) Freshly excavated fossil bones are best for amplification of ancient DNA. PNAS 104(3):739–744. doi:10.1073/pnas.0610257104

Pruvost M, Schwarz R, Correia VB, Champlot S, Grange T, Geigl EM (2008) DNA diagenesis and palaeogenetic analysis: critical assessment and methodological progress. Palaeogeogr Palaeoclimatol Palaeoecol 266(3–4):211–218. doi:10.1016/j.palaeo.2008.03.041:

Pruvost M, Bellone R, Benecke N, Sandoval-Castellanos E, Cieslak M, Kuznetsova T, Morales-Muñiz A, O'Connor T, Reissmann M, Hofreiter M, Ludwig A (2011) Genotypes of predomestic horses match phenotypes painted in Paleolithic works of cave art. PNAS 108(46):18626–18630. doi:10.1073/pnas.1108982108

R Development Core Team (2014) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www.R-project.org

Reiche I, Favre-Quattropani L, Vignaud C, Bocherens H, Charlet L, Menu M (2003) A multi-analytical study of bone diagenesis: the Neolithic site of Bercy (Paris, France). Meas Sci Technol 14:1608–1619

Ricaut FX, Keyser-Traqui C, Crubézy E, Ludes B (2005) STR-genotyping from medieval tooth and bone samples. Forensic Sci Int 151:31–35. doi:10.1016/j.forsciint.2004.07.001

Rollo F, Ubaldi M, Marota I, Luciani S, Ermini L (2002) DNA diagenesis: effect of envinronment and time on human bone. Anc Biomol 4(1):1–7

Sarasin F, Stehlin HG, Studer T (1918) Die steinzeitlichen Stationen des Birstales zwischen Basel und Delsberg. Neue Denkschriften Schweizerischen Naturforschenden Ges 54(2):79–290

Sawyer S, Krause J, Guschanski K, Savolainen V, Pääbo S (2012) Temporal patterns of nucleotide misincorporations and DNA fragmentation in ancient DNA. PLoS ONE 7(3):e34131. doi:10.1371/journal.pone.0034131

Schlumbaum A, Edwards CJ (2013) Ancient DNA research on wetland archaeological evidence. In: Menotti F, O'Sullivan A (eds) The Oxford handbook of Wetland archaeology. Oxford University Press, Oxford, pp 569–584

Schlumbaum A, Campos PF, Volken S, Volken M, Hafner A, Schibler J (2010) Ancient DNA, a Neolithic legging from the Swiss Alps and the early history of goat. J Archaeol Sci 37:1247–1251. doi:10.1016/j.jas.2009.12.025

Schwarz C, Debruyne R, Kuch M, McNally E, Schwarcz HP, Aubrey AD, Bada J, Poinar H (2009) New insights from old bones: DNA preservation and degradation in permafrost preserved mammoth remains. Nucleic Acids Res 37(10):3215–3229

Sedlmeier J (1993) Steinzeitliche Funde aus der Kohlerhöhle im Laufental. Archäol Schweiz 16(2):40–45

Smith CI, Chamberlain AT, Riley MS, Cooper A, Stringer CB, Collins MJ (2001) Neanderthal DNA: not just old but old and cold? Nature 410(6830):771–772. doi:10.1038/35071177

Smith CI, Chamberlain AT, Riley MS, Stringer C, Collins MJ (2003) The thermal history of human fossils and the likelihood of successful DNA amplification. J Hum Evol 45(3):203–217. doi:10.1016/S0047-2484(03)00106-4

Stiller M, Baryshnikov G, Bocherens H, Grandal d'Anglade A, Hilpert B, Münzel SC, Pinhasi R, Rabeder G, Rosendahl W, Trinkaus E, Hofreiter M, Knapp M (2010) Withering away—25,000 years of genetic decline preceded cave bear extinction. Mol Biol Evol 27(5):975–978. doi:10.1093/molbev/msq083

Vives S, Gilbert M, Arenas C, Gigli E, Lao O, Lalueza-Fox C (2008) Statistical analysis of post mortem DNA damage-derived miscoding lesions in Neandertal mitochondrial DNA. BMC Res Notes 1(40). doi:10.1186/1756-0500-1-40

Weinstock J, Willerslev E, Sher A, Tong W, Ho SYW, Rubenstein DI, Storer J, Burns JA, Martin LD, Bravi C, Prieto A, Froese D, Scott E, Xulong L, Cooper A (2005) Evolution, systematics and phylogeography of Pleistocene horses in the New World: a molecular perspective. PLoS Biol 3(8):e241

Willerslev E, Cappellini E, Boomsma W, Nielsen R, Hebsgaard MB, Brand TB, Hofreiter M, Bunce M, Poinar HN, Dahl-Jensen D, Johnsen S, Steffensen JP, Bennike O, Schwenninger J-L, Nathan R, Armitage S, de Hoog C-J, Alfimov V, Christl M, Beer J, Muscheler R, Barker J, Sharp M, Penkman KEH, Haile J, Taberlet P, Gilbert MTP, Casoli A, Campani E, Collins MJ (2007) Ancient biomolecules from deep ice cores reveal a forested southern Greenland. Science 317(5834):111–114. doi:10.1126/science.1141758

Xu X, Anarson U (1994) The complete mitochondrial DNA sequence of the horse, Equus caballus: extensive heteroplasmy of the control region. Gene 148(2):357–362

Electronic supplementary material

ESM 1: Details of sampled Palaeolithic sites in Switzerland with at least one equid remain. Given are number as in fig. 1, name, location, type of site, year of excavation, dating and references. Dates from this study from sampled equids, dates from the literature on various remains from the same layers as the equid remains studied here.
[a]Bodenforschung Basel-Stadt, [b]Leesch and Müller (2012), [c]Miller (2012), [d]Albrecht (1982), [e]Napierala (2008), [f]Housley *et al.* (1997), [g]Affolter *et al.* (1997), [h]INQUA-database, http://ees.kuleuven.be/geography/projects/14c-palaeolithic/ (last update June 2010):

	Site	Location			Type of site	Year of excavation	Age		Reference (first and latest)
		alt.	lat.	long.			Uncalibrated ^{14}C dates BP (own study if not stated) or layer assignment	Calendar years BP (Danzeglocke *et al.* 2012)	
1	Riehen-Ausserberg	335m	47.57	7.65	Open, dry	1967	ETH-30798 43300±900[a] ETH-30799 41760±800[a]	46937±1716 45264±944	Schmid (1967, 1976)
2	Münchenstein-Steinbruch	355m	47.51	7.62	Open, dry	1919 *et seq.*	Pre-LGM (faunal indicators)		Schaub and Jagher (1945); Furger (1977)
3	Allschwil-Ziegelei	312m	47.55	7.55	Open, dry	1923	Pre-LGM (geological indicators)		Bosinski (1967)
4	Schalberghöhle	425m	47.47	7.57	Cave	1926-27	ETH-39764 35995±320 ETH-39763 34980±330 ETH-49373 32935±268 ETH-49374 10544±41	41344±296 40032±856 37414±676 12511±140	Vogt (1936)
5	Kohlerhöhle	378m	47.43	7.57	Cave	1934-38	ETH-44380 19970±70 ETH-44381 19810±65 ETH-44382 19730±75 ETH-44383 19615±75 ETH-44379 19305±75 ETH-43310 16205±55[b] B-4969 12820±160[c] ETH-39761 12790±45 ETH-44378 12765±40 ETH-39762 12465±40 ETH-43309 12460±45[b] B-4971 11640±150[c] ETH-39760 11535±60	23897±315 23721±275 23610±251 23406±338 23067±265 19350±289 15401±471 15270±248 15223±258 14761±293 14751±297 13533±188 13423±117	Lüdin (1938); Sedlmeier (1993)
6	Kesslerloch	440m	47.75	8.69	Cave	1874/1883/1898-99/1902-03	OxA-10238 14330±110[e] OxA-10298 15020±180[e] OxA-5749 14150±100[f] OxA-10239 13980±110[e] KIA-11828 13858±55[e] ETH-44385 13690±50 OxA-5750 13670±100[f] OxA-5747 13430±100[f]	18254±263 17505±261 17392±254 17234±228 17085±180 16824±201 16745±270 16367±427	Merk (1875); Napierala (2008)

							OxA-5746	13120±90[f]	16038±40	
							KIA-11827	13052±53[e]	15941±390	
							ETH-44387	13035±60	15915±396	
							B-3329	12970±180[d]	15798±512	
							Hv-10652	12890±90[g]	15595±380	
							KIA-11829	12897±53[e]	15591±329	
							ETH-44384	12885±65	15560±333	
							ETH-44386	12795±55	15277±254	
							KIA-11825	12774±54[e]	15237±262	
							OxA-5748	12770±90[f]	15213±302	
							ETH-44388	12605±50	14962±295	
							KIA-11826	12502±52[e]	14819±295	
							KIA-33351	12335±45[e]	14502±338	
							KIA-33350	12225±45[e]	14277±239	
							B-3327	11220±180[d]	13120±198	
7	Hauterive-Champréveyres	428m	47	6.97	Open, wetland	1983-86	UCLA-2760	17695±210[c]	21128±210	Egloff (1985); Leesch *et al.* (2010)
							UZ-2285	13050±155[c]	15937±460	
							UZ-2283	12950±155[c]	15766±490	
							UZ-2286	12870±135[c]	15559±459	
							UZ-2282	12825±155[c]	15414±462	
							OxA-20700	12815±65[b]	15315±257	
							OxA-20701	12805±75[b]	15293±269	
							UZ-2171	12730±135[c]	15126±367	
							UZ-2175	12630±130[c]	14983±349	
							UZ-2172	12620±145[c]	14967±362	
							UZ-2177	12600±145[c]	14939±361	
							UZ-2170	12550±130[h]	14870±350	
							UZ-2173	12540±140[c]	14852±362	
							UZ-2174	12510±130[c]	14811±357	
							UZ-2287	12500±145[c]	14778±387	
							UZ-2284	12120±170[h]	14190±333	
							B-4530	11120±110[c]	13027±151	
8	Käsloch	420m	47.37	7.91	Cave	1905	ETH-39770	13760±45	16931±154	Bally (1908); Zuberbühler Koch (2002)
							ETH-39769	12505±45	14825±291	
							ETH-39771	12450±45	14719±313	
9	Monruz	428m	47	6.96	Open, wetland	1989-92	ETH-6412	13970±110[g]	17225±228	Egloff (1991); Leesch *et al.* (2010)
							ETH-6413	13330±110[g]	16255±436	
							ETH-6421	13140±120[g]	16064±419	

							ETH-6420 ETH-6416 OxA-20699 ETH-6417 ETH-6415 ETH-6419 ETH-6414 ETH-20727	13120±120[g] 13070±130[g] 13055±60[b] 13030±120[g] 12900±120[g] 12880±120[g] 12840±120[g] 12800±85[g]	16040±419 15970±433 15946±392 15910±435 15654±452 15586±436 15444±397 15281±282	
10	Schweizersbild	472m	47.72	8.64	Abri	1891-93	OxA-5745 ETH-44392 ETH-44390 ETH-44391 OxA-5744	13940±100[h] 12690±50 12240±60 12240±50 11780±90[h]	17196±223 15077±33 14308±258 14303±251 13678±152	Nüesch (1892); Höneisen and Peyer (1994)
11	Abri Neumühle	520m	47.44	7.33	Abri	1965	ETH-44393 B-4665	12305±40 10250±700[h]	14439±324 11789±912	Bandi (1967/68); Sedlmeier (1989)
12	Birseck-Ermitage	357m	47.49	7.63	Cave	1910/1914	B-4261 ETH-43307 B-4260	12040±80[c] 11900±55[b] 11860±100[c]	14049±239 13799±148 13776±163	Sarasin *et al.* (1918); Sedlmeier (1989)
13	Rislisberghöhle	488m	47.3	7.7	Cave	1971/1973	ETH-42517 ETH-42515 ETH-42516 ETH-42514 ETH-44377 Ly-1099 ETH-39768	13000±50[b] 12710±45[b] 12680±45[b] 12235±45[b] 12575±55 11860±230[c] 10770±45	15856±398 15112±296 15064±301 14292±245 14924±293 13868±346 12749±50	Barr (1977); Stampfli *et al.* (1983)
14	Brügglihöhle	370m	47.44	7.56	Cave	1940/1943/1951-52	Late Magdalenian (typology)			Bandi *et al.* (1952/53); Tillet (2001)

References

Affolter J, Cattin M-I, Leesch D, Morel P, Plumettaz N, Thew N, Wendling G (1997) Monruz - Une nouvelle station magdalénienne au bord du lac de Neuchâtel. Archäologie Schweiz 17(3):94-104

Albrecht G (1982) Rezension von Bandi HG et al. (1977): Die Kultur der Eiszeitjäger aus dem Kesslerloch. Jahrbuch der Schweizer Gesellschaft für Ur- und Frühgeschichte 65:239-240

Bally E (1908) Höhlenfunde im sog. Käsloch (Kt. Solothurn). Anzeiger für schweizerische Altertumskunde 10:1-12

Bandi HG (1967/68) Untersuchung eines Felsschutzdaches bei Neumühle, Gemeinde Pleigne, Kt. Bern. Jahrbuch des Bernschen historischen Museums 47/48:95-113

Bandi HG, Lüdin C, Mamber W, Schaub S, Schmid E, Welten M (1952/53) Die Brügglihöhle an der Kohlholzhalde bei Nenzlingen (Kt. Bern), eine neue Fundstelle des Spätmagdalénien im untern Birstal. Jahrbuch des Bernschen historischen Museums 32/33:45-76

Barr JH (1977) Die Rislisberghöhle, ein neuer Magdalénien-Fundplatz im Schweizer Jura. Archäologisches Korrespodenzblatt 7 (2):85-87

Bosinski G (1967) Die mittelpaläolithischen Funde im Westlichen Mitteleuropa. Köln, Graz, Böhlau

Danzeglocke U, Jöris O, Weniger B (2012) CalPal-2007 online. Accessed dec. 2012-march 2013

Egloff M (1985) Fouilles de Champréveyres. Musée Cantonal d'Archéologie:109-119

Egloff M (1991) Rapport Annuel. Musée cantonal d'Archéologie

Furger R (1977) Die mittelpaläolithische Station beim untern Steinbruch von Münchenstein BL. Regio Basilensis:58-72

Höneisen M, Peyer S (1994) Schweizersbild – ein Jägerlager der Späteiszeit: Beiträge und Dokumente zur Ausgrabung vor 100 Jahren. Kantonsarchäologie, Schaffhausen

Housley R, Gamble C, Street M, Pettitt P (1997) Radiocarbon evidence for the Lateglacial Human Recolonisation of Northern Europe. Proceedings of the Prehistoric Society 63:25-54

Leesch D, Bullinger J, Cattin M, Müller W, Plumettaz N (2010) Hearth and hearth-related activities in Magdalenian open-air sites: the case studies of Champréveyres and Monruz (Switzerland) and their relevance to an understanding of upper Palaeolithic site structure. In: The Magdalenian in central Europe. New finds and concepts. Instytut Archeologii Uniwersytetu Rzeszowskiego, Rzeszów pp 53-69

Leesch D, Müller W (2012) Neue Radiokarbondaten an Knochen, Zähnen und Geweih aus Magdalénien-Fundstellen der Schweiz und ihre Bedeutung für die Stellung des Magdalénien innerhalb des Spätglazials. Jahrbuch der Archäologie Schweiz 95:1-12

Lüdin C (1938) Kohlerhöhle bei Brislach (Amt Laufen, Bern). Jahrbuch der Schweizerischen Gesellschaft für Ur- und Frühgeschichte 30:61-64

Merk C (1875) Der Höhlenfund im Kesslerloch bey Thayngen (Kanton Schaffhausen): Originalbericht des Entdeckers. Hans Staub, Zürich

Miller R (2012) Mapping of the Northwest Magdalenian. Quatern Int 272/273:209-230

Napierala H (2008) Die Tierknochen aus dem Kesslerloch. Neubearbeitung der paläolithischen Fauna, vol 2. Beiträge zur Schaffhauser Archäologie. Baudepartement des Kantons Schaffhausen, Kantonsarchäologie, Schaffhausen

Nüesch J (1892) Une station préhistorique à Schweizersbild près de Schaffhouse. Archives des sciences physiques et naturelles:541-548

Sarasin F, Stehlin HG, Studer T (1918) Die steinzeitlichen Stationen des Birstales zwischen Basel und Delsberg. Neue Denkschriften der Schweizerischen Naturforschenden Gesellschaft 54 (2):79-290

Schaub S, Jagher A (1945) Zwei neue Fundstellen von Höhlenbär und Höhlenhyäne im unteren Birstal. Ecologae geologicae Helvetiae:621-634

Schmid E (1967) Archäologische Bodenforschung des Kantons Basel-Stadt: Riehen-Ausserberg. Basler Zeitschrift für Geschichte und Altertumskunde 67:30-34

Schmid E (1976) Beobachtungen an würmeiszeitlichen Hyänenkoprolithen und zerbissenen Knochen. Thèmes specialises:143-147

Sedlmeier J (1989) Jungpaläolithikum und Spätpaläolithikum in der Nordwestschweiz. Ein Beitrag zur regionalen Erforschung des Paläolithikums auf Grund ausgewählter Fundinventare aus Grabungen zwischen 1910 und 1956. Bern

Sedlmeier J (1993) Steinzeitliche Funde aus der Kohlerhöhle im Laufental. Archäologie Schweiz 16 (2):40-45

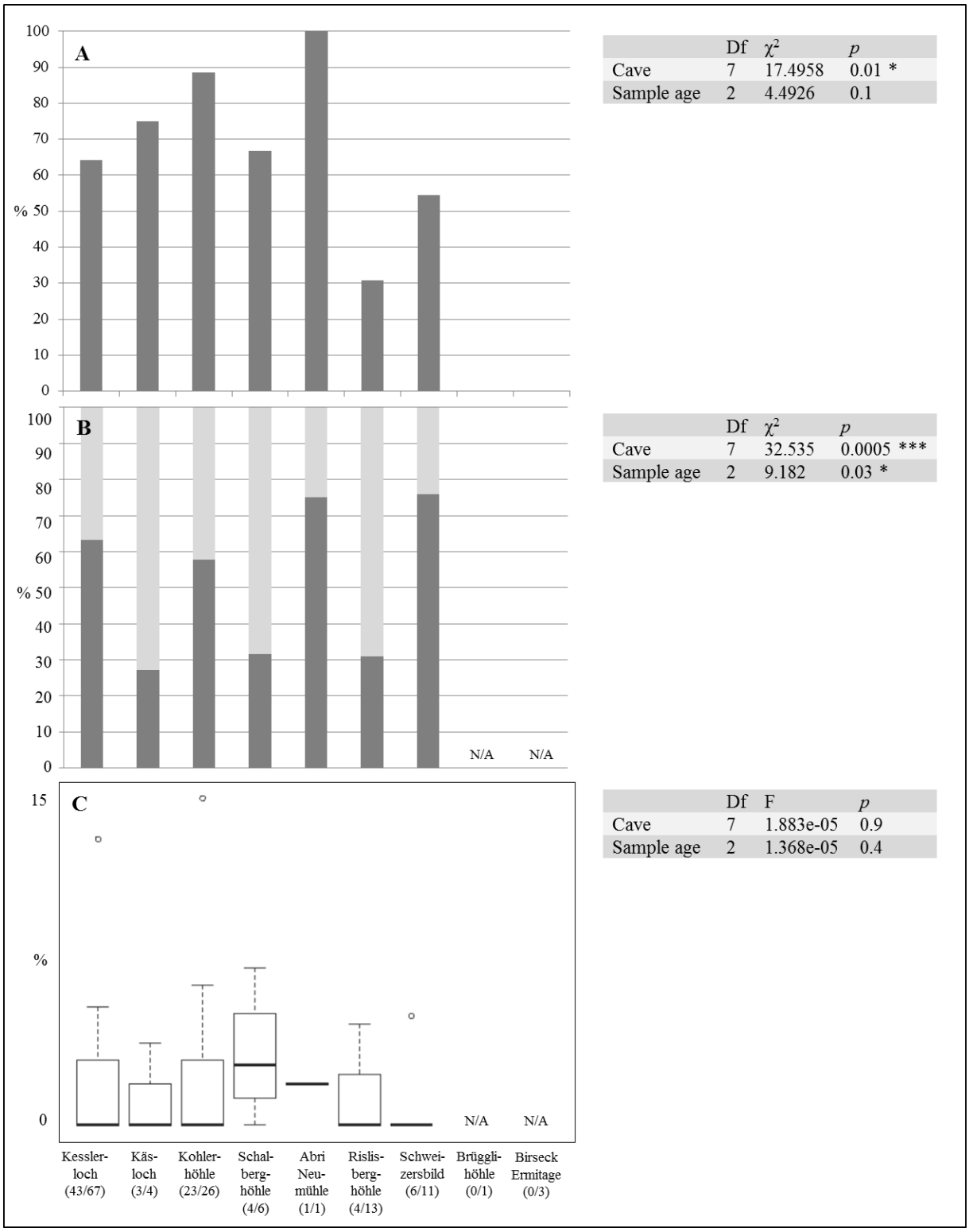
Stampfli HR, Barr JH, Glutz R, Joos M, von Koenigswald W, Lepiksaa J, Müller E, Müller M, Wegmüller S, Wiesli U (1983) Rislisberghöhle. Archäologie und Ökologie einer Fundstelle aus dem Spätmagdalénien bei Oensingen im Solothurner Jura. Academica Helvetica. Paul Haupt, Bern, Stuttgart

Tillet T (2001) Les Alpes et le Jura: quaternaire et préhistorique ancienne. Éditions scientifiques. Paris

Vogt E (1936) Die paläolithische Station in der Höhle am Schalbergfelsen. Denkschriften der Schweizerischen Naturforschenden Gesellschaft 71 (3):1-70

Zuberbühler Koch K (2002) Die magdalénienzeitliche Höhlenstation Winznau/Käsloch im Kanton Solothurn. Bern

ESM 2: Effect of tissue type (tooth, bone), storage time after excavation (20-50 y, 70-90 y, 110-140 y); and sample age (17-12 ky, 23 ky, 47-37 ky) on: A: percentage of samples with amplifiable 75 bp mtDNA d-loop fragment; B: proportion of positive (dark grey) to negative (light grey) PCR products; C: mtDNA damage as represented by percentage of C→T substitutions for positive Palaeolithic equid remains from cave/abri sites in Switzerland. See fig. 2, 3, and 5 for the graphical descriptions (charts) of samples ages. Significance codes: 0.001 = ***, 0.01 = **, 0.05 = *.



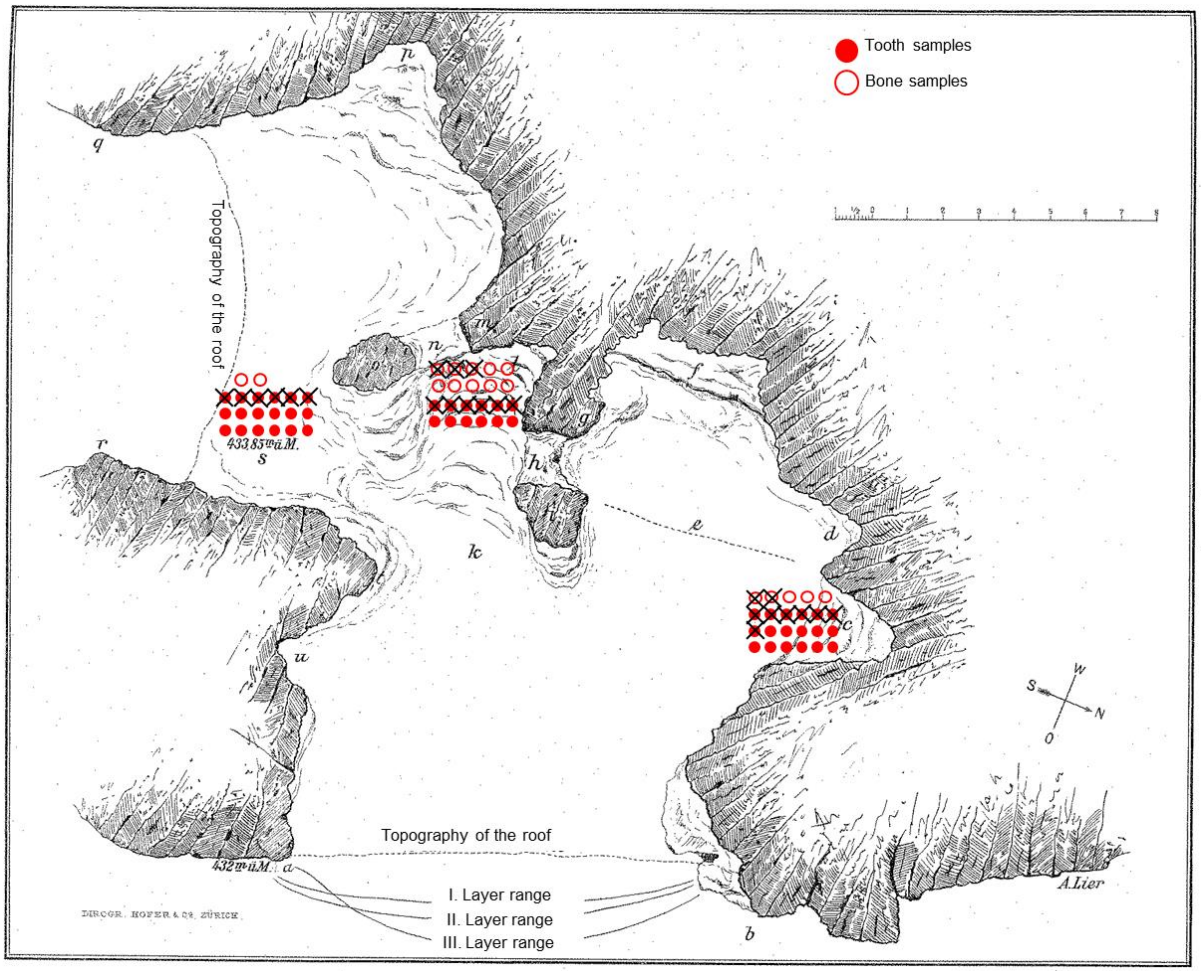
	Df	χ^2	*p*
Cave	7	17.4958	0.01 *
Sample age	2	4.4926	0.1

	Df	χ^2	*p*
Cave	7	32.535	0.0005 ***
Sample age	2	9.182	0.03 *

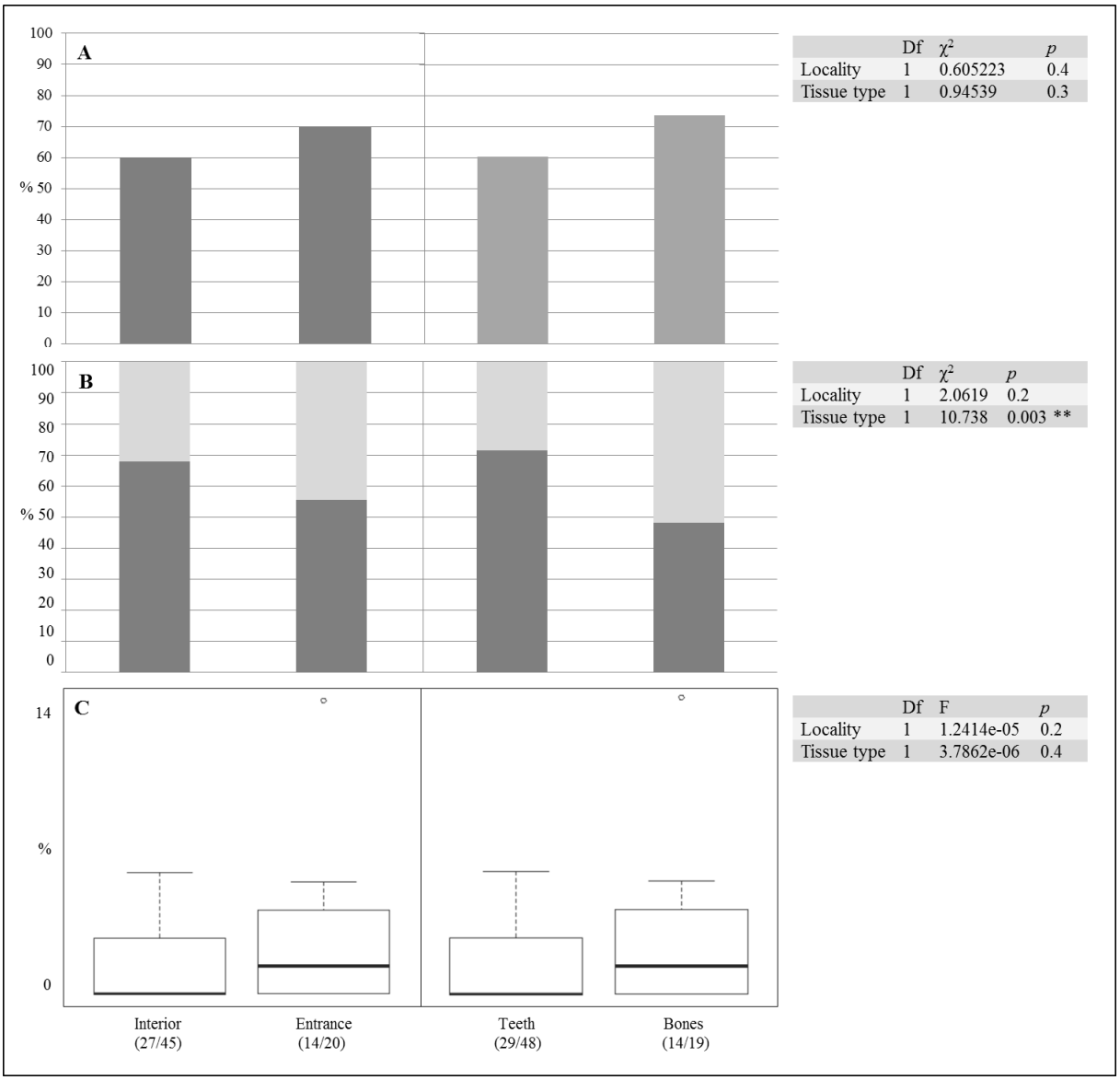
	Df	F	*p*
Cave	7	1.883e-05	0.9
Sample age	2	1.368e-05	0.4

ESM 3: Horizontal plan of Kesslerloch, Switzerland with Palaeolithic equid samples from the interior (section n-m, and c) and from the entrance area (s). Samples without amplifiable 75 bp mtDNA d-loop fragment are crossed. Two samples from “general area” are not shown. Modified from Heierli (1907).

Heierli J (1907) Das Kesslerloch bei Thaingen. Neue Denkschriften der Schweizerischen Naturforschenden Gesellschaft 43



ESM 4: Effect of distribution within the cave (interior, entrance) and tissue type (tooth, bone) on: A: percentage of samples with amplifiable 75 bp mtDNA d-loop fragment; B: proportion of positive (dark grey) to negative (light grey) PCR products; C: mtDNA damage as represented by percentage of C→T substitutions for positive Palaeolithic equid remains from Kesslerloch, Switzerland. Significance codes: 0.001 = ***, 0.01 = **, 0.05 = *.

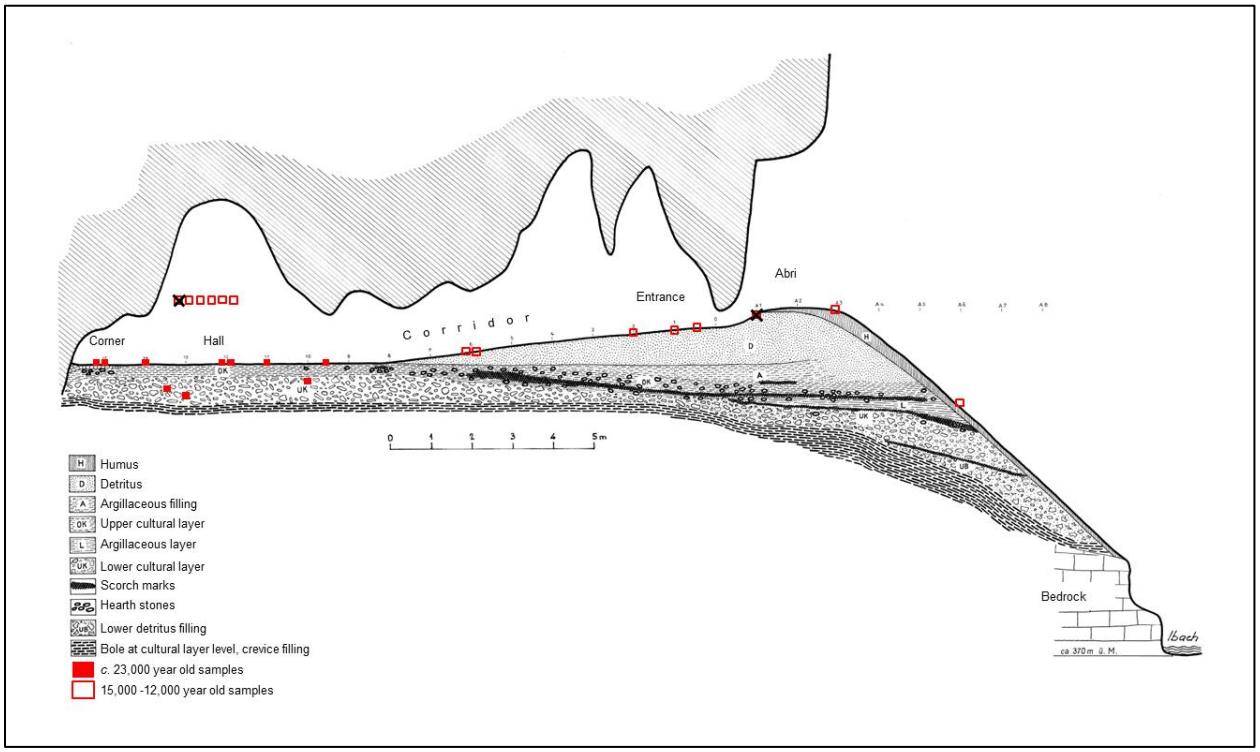


	Df	χ^2	*p*
Locality	1	0.605223	0.4
Tissue type	1	0.94539	0.3

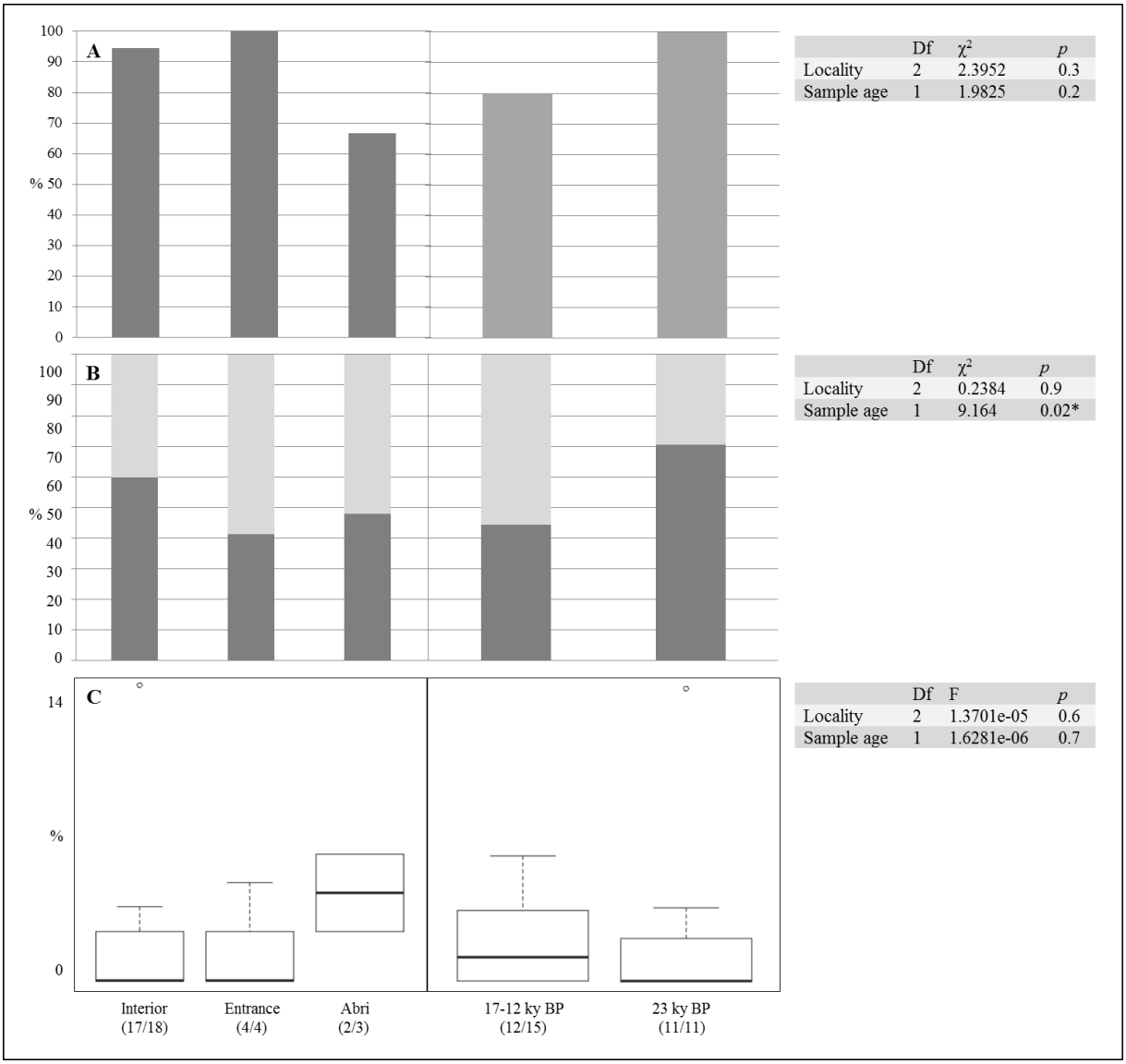
	Df	χ^2	*p*
Locality	1	2.0619	0.2
Tissue type	1	10.738	0.003 **

	Df	F	*p*
Locality	1	1.2414e-05	0.2
Tissue type	1	3.7862e-06	0.4

ESM 5: Longitudinal section of Kohlerhöhle, Switzerland showing location of the samples in the abri (Abri), entrance (Entrance) and the interior (Corridor, Hall, Corner). Samples without amplifiable mtDNA are crossed. Two samples without known location are not shown. Modified with permission from Archäologie und Kantonsmuseum Basel-Landschaft.



ESM 6: Effect of distribution within the cave (abri, entrance and interior) and age (17-12 ky, 23 ky) on: A: percentage of samples with amplifiable 75 bp mtDNA d-loop fragment; B: proportion of positive (dark grey) to negative (light grey) PCR products; C: mtDNA damage as represented by percentage of C→T substitutions for positive Palaeolithic equid remains from Kohlerhöhle, Switzerland. Significance codes: 0.001 = ***, 0.01 = **, 0.05 = *.



	Df	χ^2	*p*
Locality	2	2.3952	0.3
Sample age	1	1.9825	0.2

	Df	χ^2	*p*
Locality	2	0.2384	0.9
Sample age	1	9.164	0.02*

	Df	F	*p*
Locality	2	1.3701e-05	0.6
Sample age	1	1.6281e-06	0.7

2.2 Elsner J, Hofreiter M, Schibler J, Schlumbaum A (2017) Ancient mtDNA diversity reveals specific population development of wild horses in Switzerland after the Last Glacial Maximum. *PLoS ONE* 12(5): e0177458, doi:10.1371/journal.pone.0177458

PLOS ONE

RESEARCH ARTICLE

Ancient mtDNA diversity reveals specific population development of wild horses in Switzerland after the Last Glacial Maximum

Julia Elsner[1]*, **Michael Hofreiter**[2], **Jörg Schibler**[1], **Angela Schlumbaum**[1]

1 Integrative Prehistory and Archaeological Science, University of Basel, Basel, Switzerland, **2** Institute for Biochemistry and Biology, University of Potsdam, Potsdam, Germany

* Julia.Elsner@email.de

OPEN ACCESS

Citation: Elsner J, Hofreiter M, Schibler J, Schlumbaum A (2017) Ancient mtDNA diversity reveals specific population development of wild horses in Switzerland after the Last Glacial Maximum. PLoS ONE 12(5): e0177458. https://doi.org/10.1371/journal.pone.0177458

Editor: David Caramelli, University of Florence, ITALY

Received: November 30, 2016

Accepted: April 27, 2017

Published: May 24, 2017

Data Availability Statement: Data are available from GenBank (ncbi.nlm.nih.gov/genbank) with accession numbers KC893753-KC893844.

Funding: Julia Elsner was funded by Swiss National Science Foundation (K-31K1_120528/1), Freiwillige Akademische Gesellschaft Basel, and Nikolaus and Bertha Burckhardt-Bürgin-Foundation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

On large geographical scales, changes in animal population distribution and abundance are driven by environmental change due to climatic and anthropogenic processes. However, so far, little is known about population dynamics on a regional scale. We have investigated 92 archaeological horse remains from nine sites mainly adjacent to the Swiss Jura Mountains dating from c. 41,000–5,000 years BP. The time frame includes major environmental turning points such as the Last Glacial Maximum (LGM), followed by steppe vegetation, afforestation and initial re-opening of the landscape by human agricultural activities. To investigate matrilinear population dynamics, we assembled 240 base pairs of the mitochondrial d-loop. F_{ST} values indicate large genetic differentiation of the horse populations that were present during and directly after the LGM. After the retreat of the ice, a highly diverse population expanded as demonstrated by significantly negative results for Tajima's *D*, Fu's F_S and mismatch analyses. At the same time, a different development took place in Asia where populations declined after the LGM. This first comprehensive investigation of wild horse remains on a regional scale reveals a discontinuous colonisation of succeeding populations, a pattern that diverges from the larger Eurasian trend.

Introduction

Population distributions and abundance patterns of species are driven by environmental change. Natural and anthropogenic processes impact the availability of food resources for large herbivores, but species respond individually to these challenges (e.g., [1, 2]). Generally, large animals are more likely to react to climate change due to longer generation intervals and smaller effective population size [3]. The Pleistocene-Holocene transition is characterised by profound climatic and thus environmental change, and species well adapted to the open steppe vegetation in Eurasia and North America were confronted with fragmentation and even loss of habitat, yet some were able to establish new niches and survived [1, 4]. A combination of human hunting pressure and habitat fragmentation caused by climate change seems to be the

Competing interests: The authors have declared that no competing interests exist.

most appropriate explanation for most extinction events in the Old World [5–8] and is supported by both archaeological and palaeo-climatic evidence.

One of the key species of the Pleistocene steppe was the horse, *Equus ferus caballus* L. 1758. The palaeontological and archaeological record shows its abundance in an area ranging from North America to southern Europe. In the first genetic studies featuring Pleistocene horses from North America and Eurasia, it emerged that in addition to a mitochondrial clade consisting of North American horses, a second clade included both *E. f. caballus* from North America and Eurasia, and domestic horses [9, 10]. Cieslak *et al.* [11] further showed that while some matrilines were regionally confined to Alaska, the Eurasian steppe, and Iberia, others were extremely widely distributed suggesting a panmictic population. The approach of Lorenzen *et al.* [1] focused on (amongst others) horse population development in response to climate change, habitat distribution and human encroachment on a global scale, mainly featuring specimens from north-eastern Asia and north-western America. A positive correlation between available habitat size and genetic diversity supports their conclusion that climate had been the major driving force in population changes over the past 50 thousand years (ka). Horses were thriving particularly under cold and arid conditions. The authors found, however, that the drastic decline of genetic diversity in horses after the Last Glacial Maximum (LGM) could not be explained by habitat reduction alone and thus might reflect the impact of expanding human populations in Eurasia as indicated by the prevalence of horse remains in the archaeological (not palaeontological) record [1]. This scenario was supported by Orlando *et al.* [12], who found support for models indicating population reduction in the interglacial phases and expansion during the cold stages of Marine Isotope Stages (MIS) 4 and 3, followed by a 100-fold collapse after the LGM. In contrast to these large scale developments, little is as yet known about the effect of environmental change on regional horse populations, and it is probable that they responded more variably, depending on local conditions.

To investigate this issue, we focused on local horse population development through the course of c. 50 ka in the heterogeneous landscape between and including the Alps and the Jura Mountain Chain—present day Switzerland. This region was subjected to sometimes rapid environmental change. Both Alpine and Jura glaciers reached their maximal extent at c. 25 ka BP and started to retreat between 22 and 21 ka BP [13–15]. Deglaciation progressed quite rapidly; the northern Jura c. 50 km south of Basel (Rhine knee area) was ice-free by 19 ka BP [16], while around 18 ka BP, soil development had started in the Alpine foreland [17] when 80% of the LGM ice had melted [18]. Environmental conditions improved rapidly and human (temporary) settlements, first at the foot of the mountains and in caves, later towards the lakes [19], became more numerous [20]. Palynological data indicate herbaceous, heliophilous vegetation until c. 14.7 ka BP, forming grassland interspersed with dwarf shrubs [20–22]. At 14.7 ka BP, mean temperature rose by c. 5˚C [21] and in the course of the following 2,000 years the landscape turned into open woodland; by 11 ka BP, forest canopy was probably closed [23]. Anthropogenic influence (agricultural activity) becomes traceable around 7 ka BP in the Jura [21] and in the Alpine foreland [24]. Since the Neolithic, large game species have lost significance in human diets [25], but since agriculture and domestic animal husbandry demanded open landscapes, wild species were increasingly displaced. Horses are absent from the Mesolithic archaeological record in Switzerland despite numerous known sites (yet less than from earlier and later periods) which include faunal assemblages [19]. Most likely, the last wild horses in Switzerland stem from Neolithic lakeshore settlements where horses are present in very low amounts; however, it cannot be ultimately ruled out that they represent first domestics [26]. It is assumed that from the Bronze Age onwards, all horse remains stem from domestic animals [27].

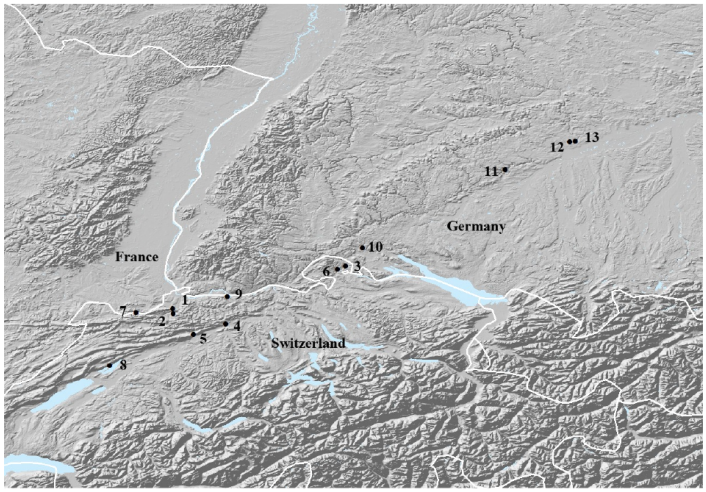


Fig 1. Map of investigated sites in Switzerland and added sites in Germany. Site numbers according to Table 1.

https://doi.org/10.1371/journal.pone.0177458.g001

We have investigated horse teeth and bones from nine sites mainly adjacent to the Swiss Jura Mountains dating from c. 41 to 5 ka BP (Fig 1, Table 1, S1 Table). The dataset comprises all known Pleistocene sites with more than one horse remain. To address matrilinear population dynamics of Upper Pleistocene and Holocene wild horses, we have assembled 240 base

Table 1. Details of the investigated sites, number of samples and haplogroups. Archaeological cultures according to Leesch [28]. Mitochondrial haplogroup nomenclature follows Cieslak *et al.* [11]. Haplogroups identified from directly dated specimens are indicated in italics. For individual dates, skeletal elements and references see S1 Table.

	Name of site	^{14}C Age (cal ka BP)	Archaeological culture	Number of positive samples	Mitochondrial haplogroups
1	Schalberghöhle	41–37	-	3	*A*; *X3*
		12.5	Azilian	1	*B*
2	Kohlerhöhle	24–23	Badegoulian	11	B; H; *X3*
		15–13.5	Magdalenian	12	*A*; *B*; D; K
3	Kesslerloch	18–14	Magdalenian	43	*A*; *B*; D; *H*; *K*; X3; X4b
4	Käsloch	17–15	Magdalenian	3	*B*; *H*
5	Rislisberghöhle	15–12.5	Magdalenian—Azilian	4	*K*
6	Schweizersbild	15–14	Magdalenian	6	*A*; *B*; *X3*
7	Abri Neumühle	14.5	Magdalenian	1	*C*
8	Twann-Bahnhof	5.5	Neolithic	6	A; *B*; D
9	Mumpf	5	Neolithic	2	*D*
10	Petersfels[a, b]	15–14	Magdalenian	2	*A*; *B*
11	Hohlefels[a]	14.8	Magdalenian	1	*C*
12	Bocksteinhöhle[a]	50	-	1	*A*
13	Vogelherdhöhle[a]	17	Magdalenian	1	*A*
				total = 97	

[a] Weinstock *et al.* [10];

[b] Lorenzen *et al.* [1]

https://doi.org/10.1371/journal.pone.0177458.t001

pairs (bp) of the mitochondrial d-loop. We aim to contextualise population developments and natural and anthropogenic changes of the environment in a fringe area of the Eurasian steppe biota. These results are compared with published data from Northern Asia and the Ural region, the heartland of the steppe.

Materials and methods

Archaeological samples

A total of 202 horse (*Equus* sp.) teeth and bones were sampled either from Palaeolithic or Neolithic anthropogenic cultural layers, or from palaeontological contexts associated with hyena hunting activity and chance finds; 92 of them yielded amplifiable mtDNA [29]. All sites are located in or close to the Jura Mountains in Switzerland (Fig 1, Table 1, S1 Table). The samples had been stored in museums and archaeological collections since their excavations. To obtain direct dates from each layer at the respective sites, 31 samples were chosen for ^{14}C dating using accelerator mass spectrometry (AMS) at ETH Zurich, Switzerland, and calibrated with CalPal [30]. Additional ^{14}C dates were assembled from the literature. In some cases the age was projected from dendrochronology or typology (S1 Table). Five ^{14}C dated sequences of Pleistocene horse remains (DQ007558/DQ007611: 14'751 cal BP, DQ007556/DQ007609: 14'752 cal BP, DQ007591: 16'928 cal BP, DQ007590: 50'735 cal BP; [10] FJ204352: 14'500 cal BP; [1] from the Swabian Jura were added to the dataset, resulting in 97 specimens to be analysed.

Processing of ancient samples

Preparation, extraction, amplification and Sanger sequencing of ancient samples were performed as described in Elsner *et al.* [29] in dedicated ancient DNA facilities following established standards for aDNA work [31], including multiple independent extractions and PCR, and routine cloning. Mitochondrial d-loop positions 15,492 to 15,669 and 15,696 to 15,758 [32] were targeted in seven partially overlapping fragments [33]. Contamination of ancient samples was never detected; all PCR amplification products in the extraction and PCR blank controls came from microorganisms or were unidentifiable according to GenBank Blast search and are most likely explained by the permissive PCR set up (low annealing temperature, up to 70 cycles).

Data analysis

Sequences were edited and aligned by eye with BioEdit [34]. A consensus sequence was built from at least three amplifications from a minimum of two independent extractions based on majority. To deal with sequences with missing nucleotides on the one hand, and to avoid specimens that stemmed from potentially mixed-up layers and an overrepresentation of Magdalenian samples, the analyses were done on three datasets (Table 2).

Table 2. Datasets used for analyses.

	Selection criterion	Number of sequences						
		Total	Palaeontological	Badegoulian	Madgalenian	Magd. +Azilian	Azilian	Neolithic
Dataset 1	all samples	**97**	4	11	70	74	4	8
Dataset 2	samples with > 40% missing nucleotides excluded	**78**	4	11	53	57	4	6
Dataset 3	only ^{14}C dated samples	**36**	4	5	20	-	3	4

https://doi.org/10.1371/journal.pone.0177458.t002

Within the datasets, samples were assembled into time bins according to similar environmental conditions. Magdalenian and Azilian samples were handled both in combination and separately because, on the one hand, the transition between the cultural horizons is marked by the temperature increase at c. 14.7 ka BP, but, on the other hand, possibly mixed up layers within the sites may have led to mis-assignment of individual specimens. The combined category could thus be dropped for dataset 3. Both Azilian and Neolithic date into the early Holocene, yet we assume different dynamics in the Neolithic due to anthropogenic interference to the landscape leading to a dilution of the interdependence of latitude/temperature and vegetation, and thus did not combine those samples.

Nucleotide diversity is defined as the average number of nucleotide differences per site between two DNA sequences in all possible pairs in the population studied, while haplotype diversity is a way to describe the uniqueness of a haplotype in a population. These indices as well as F_{ST} values and tests to detect recent population expansion (Tajima's *D*, Fu's *FS*, sum of squared deviations SSD, Harpending's raggedness index) were computed with Arlequin 3.5 [35] with missing data coded as '?' and allowed level of missing data set to 5%. Tajima's *D* [36] uses the mean average number of pairwise nucleotide differences and the number of segregating sites, each scaled so that they are expected to be the same in a neutrally evolving population of constant size; Fu's F_S [37] is based on the number of alleles (haplotypes). It is generally assumed that Fu's F_S is more sensitive in detecting population expansion than Tajima's *D*. The raggedness index [38, 39] is also used to detect recent population expansions, which is rejected by non-significant results. The SSD between observed and expected mismatch (distribution of the number of sequence differences) quantifies the smoothness of the observed mismatch.

To reject a statistical bias in the analyses introduced by uneven sample sizes in the respective time bins, directly compared time bins (e.g. Magdalenian and LGM) were randomized (10k permutations with replacement) using nucleotide and haplotype diversity estimated with the packages *pegas* [40] and *seqinR* [41] implemented in R [42] using the option 'pairwise deletion of missing data'. This was done by pooling the sequences of two time bins, repeatedly creating two pseudo-groups of the same size of the original bins from the pool and collecting diversity parameters from them. In case the combined pseudo diversity deviated from the original diversity (threshold 0.05), sampling bias has to be assumed.

For the construction of Median Joining Networks (MJN) [43] with the program Network (fluxus-engineering.com) polymorphic nucleotide positions were down-weighted according to the number of polymorphisms (default 50) [33]. The transition: transversion weight was set to 1: 10 (S2 Table). Principal component analysis (PCA) was based on relative haplogroup frequencies within the Eurasian dataset and computed with PAST [44].

Results

Swiss and Swabian wild horse populations

The maximal sequence length of 241 bp (without primers) could be assembled for 50 specimens, 231 bp for 15 samples. The remaining 27 specimens had missing data mostly between positions 15,564 and 15,669 relative to the horse reference mitogenome sequence [32]. Amongst the 92 Swiss and five Swabian horse samples, 36 polymorphic sites were present resulting in 41 haplotypes (ht), which can be summarized into eight haplogroups (hg) according to Cieslak *et al.* [11]: A, B, C, D; H, K and X3 (Fig 2; S3 Table). One previously unnamed haplogroup was labelled X4b following [11], distinguished by nucleotide positions 15495, **15540**, 15602, (**15718**) and 15720 (defining nucleotide positions in bold, optional position in parenthesis).

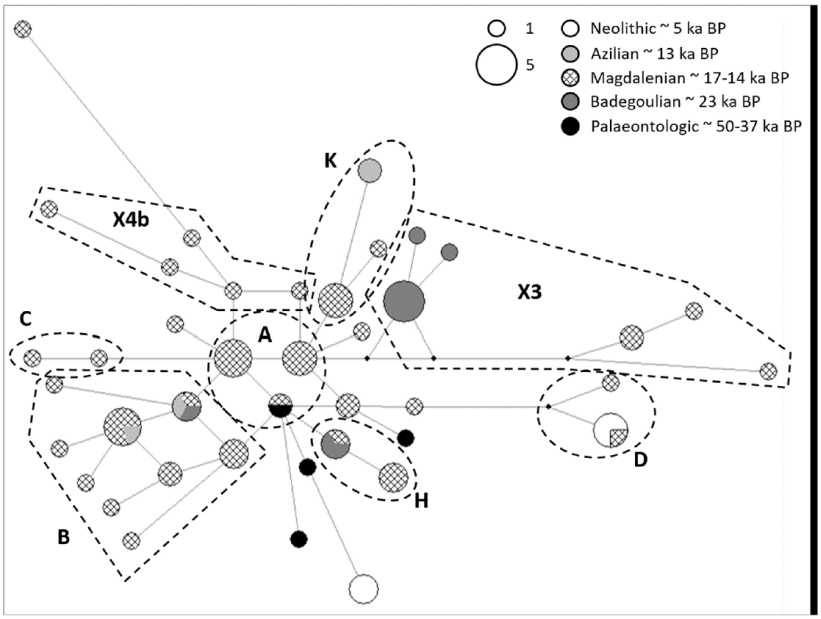


Fig 2. Median Joining Network (MJN) of horse populations (97 samples, max. 241 bp). Nodes are proportional to frequencies and branch length according to number of substitutions. Haplogroup nomenclature follows Cieslak *et al.* [11].

https://doi.org/10.1371/journal.pone.0177458.g002

Nucleotide and haplotype diversities are shown in Table 3 (see also S4 Table). Both nucleotide and haplotype diversities are highest in the Magdalenian and lowest in the Badegoulian. Note that nucleotide diversity of the Neolithic deme is relatively high compared to the lower haplotype diversity, indicating population fragmentation.

The Median Joining Network (241 bp) shows only little haplotype continuity between the time bins. Two lineages (hgs B and H) are found in Switzerland during and after the LGM, and two lineages of hg B continue into the Azilian (Figs 2 and 3). All other haplotypes are restricted to single time bins or at least do not occur in succeeding time bins. For reasons of comparability, we tested whether a bias was introduced by uneven sample sizes within the time bins. This can be rejected for the comparison between Magdalenian (+ Azilian) and Badegoulian by permutation testing (S1 Fig). F_{ST} values for these time bins portend great genetic differentiation (0.17, $p < 0.001$) (S5 Table).

Table 3. Nucleotide and haplotype diversities in horse populations from Switzerland and the Swabian Jura (dataset 2).

Time period	Number of samples	Number of haplotypes	Nucleotide diversity	Haplotype diversity
Palaeontological	4	4	0.0104	1
Badegoulian	11	4	0.0093	0.6
Magdalenian	53	28	0.016	0.95
Magd. + Azilian	57	29	0.0159	0.95
Azilian	4	3	0.0104	0.83
Neolithic	6	2	0.0149	0.6

https://doi.org/10.1371/journal.pone.0177458.t003

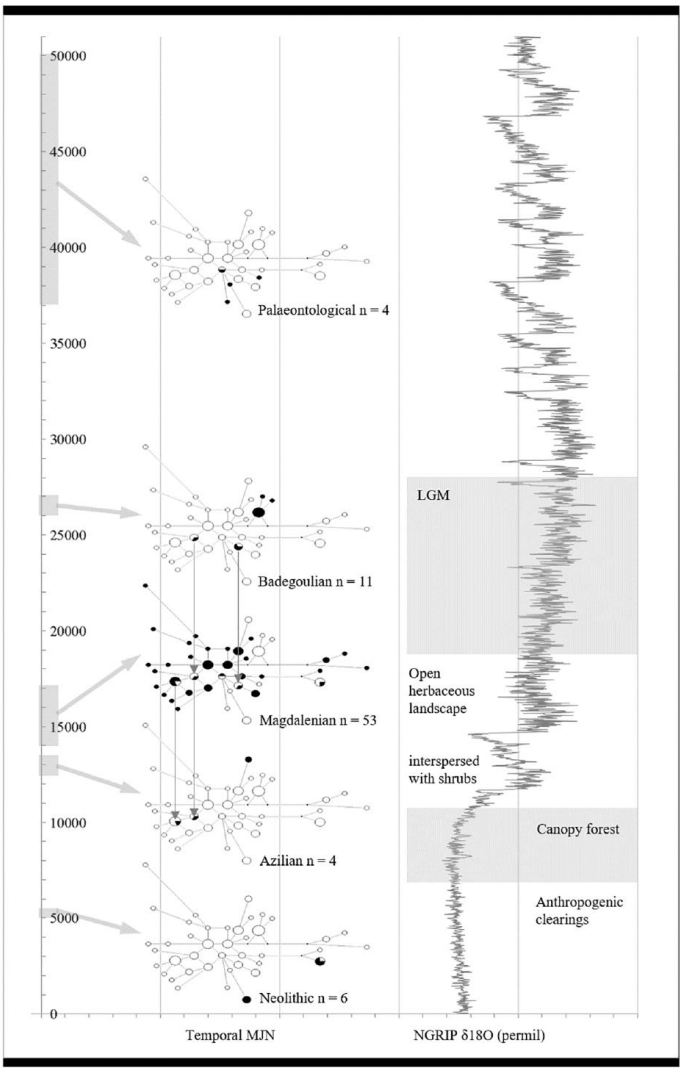


Fig 3. Temporal Median Joining Networks (MJN) of Swiss and Swabian horse populations (97 samples, max. 241 bp) in the context of environmental conditions and temperature. Light grey boxes and arrows show the age of the sequences the particular MJN is based on. Empty nodes represent haplotypes absent from particular time bin. Vertical arrows indicate continuity of haplotypes from subsequent time bins. $\delta^{18}O$ record of the North Greenland Ice Core Project (NGRIP) after [49–53], vegetation data from [21, 54].

https://doi.org/10.1371/journal.pone.0177458.g003

Simulations according to Tajima [36] and Fu [37] indicate recent population expansion for the Magdalenian and the combined Magdalenian and Azilian sample sets (Table 4, S6 Table). This is supported by the distribution of the number of sequence differences between haplotypes (mismatch analysis, S2 Fig). Both the palaeontological and Magdalenian (+ Azilian) bins exhibit unimodal distributions. Equally, neither the sum of the squared deviations (SSD) nor

Table 4. Tajima's *D*, Fu's F_S, sum of squared deviations (SSD) and Harpending's raggedness index results for horse populations from Switzerland and the Swabian Jura (dataset 2). Significant results are in bold.

Time period	Tajima's *D*	*p*	Fu's F_S	*p*	SSD	*p*	Raggedness index	*p*
Palaeontological	-0.8	.2	-1.51	.06	0.02	0.8	0.1	0.9
Badegoulian	-0.09	.5	0.77	.7	0.13	0.1	0.3	0.1
Magdalenian	-1.32	.07	**-21.71**	0	0.001	0.6	0.02	0.6
Magd. + Azilian	-1.29	.08	**-22.59**	0	0.001	0.5	0.02	0.6
Azilian	1.37	.9	0.46	.5	0.09	0.3	0.25	0.7
Neolithic	2.12	1	4.51	1	**0.38**	0.02	**0.88**	0.03

https://doi.org/10.1371/journal.pone.0177458.t004

Harpending's raggedness index [38, 39] were statistically significant, further indicating population expansion (Table 4). This applies for the Badegoulian and Azilian time bins as well. However, an increased SSD and raggedness as well as multimodal distribution point to populations with secondary contact, i.e. populations that have received genetic input from further populations [45]. For the Neolithic time bin, population expansion can be rejected explicitly; the very high SSD and raggedness index might even indicate that individuals from the same site stemmed originally from independent populations.

In Fig 3, a temporal Median Joining Network (MJN) of 97 sequences (up to 241 bp) from the Swiss and Swabian Jura is put into context with the $\delta^{18}O$ record of the North Greenland Ice Core Project (NGRIP) and prevailing regional environment. During the LGM, three distinct haplogroups are present (all from one site), two of which are also found in the Magdalenian. Most lineages present during the LGM did not reoccur in later time periods. The dominating haplotype X3 has as yet not been detected in Eurasian Pleistocene horses [1, 10–12] but is frequent in some modern breeds, both in Iberia (e.g. [46]) and Asia (e.g. [47, 48]). During the Magdalenian, when an open herbaceous landscape prevailed, the network topology shows a star-like expansion pattern. After c. 14.7 ka BP when global atmospheric temperature rose by c. 5°C and forestation began, only two genetically distant lineages are recovered from the Azilian. By c. 12 ka BP temperatures levelled off at present day conditions. Two distinct matrilines, one of which occurred in the Magdalenian, are present in the Neolithic when early human impact on the environment is observed. Generally, very little continuity existed through time.

Comparison with Pleistocene horse sequences from across Eurasia

The Swiss and Swabian Jura sequences were put into context with published samples from Northern Asia and the Urals regions (S7 Table). They were sorted into time bins: before the LGM (BLGM, c. 50–27.5 ka BP), during the LGM (LGM, c. 25–22.5 ka BP), and after the LGM (PLGM, c. 18–12.5 ka BP). Nucleotide diversity is highest in Asia at all times, and is decreasing westwards (Fig 4). During the LGM, it drops slightly and rises to higher level afterwards in Asia and Switzerland, yet decreases in the Ural region. Haplotype diversity has to be regarded with caution due to low sample sizes (see Fig 5, S7 Table). Generally the trend seen from nucleotide diversity is repeated (Fig 4, S8 Table).

Because the sample sizes in the Eurasian datasets are low compared to the Swiss/Swabian Jura, an unbiased comparison of F_{ST} values is only possible for the time bins Swiss/Swabian LGM and Swiss/Swabian PLGM (see above) and Swiss/Swabian LGM and Asian BLGM (F_{ST} 0.23, $p < 0.001$; S3 Fig, S9 Table). A principal component analysis (PCA) based on relative haplogroup frequencies demonstrates that while the Asian samples are genetically close to each other through time, and might, together with the Ural BLGM lineages, be addressed as

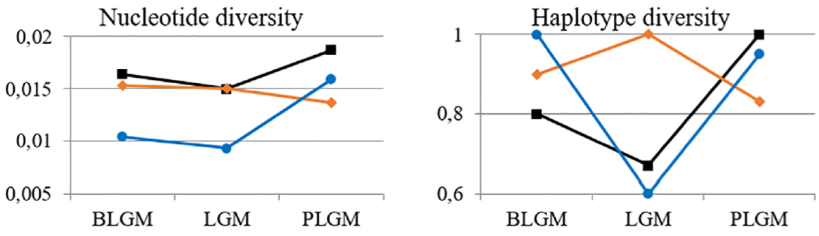


Fig 4. Nucleotide (left panel) and haplotype (right panel) diversity in Asia (black squares), Ural region (red diamonds) and the Swiss and Swabian Jura (blue circles) before (BLGM), during (LGM) and after (PLGM) the Last Glacial Maximum.

https://doi.org/10.1371/journal.pone.0177458.g004

panmictic, the Swiss/Swabian samples are more distinct. Moreover, the interruptive nature of the LGM concerning population continuity is apparent as the LGM demes from all regions stand apart from the preceding and succeeding populations (Fig 5, S4 Fig).

Discussion

The investigation of mt d-loop sequences from 97 horse teeth and bones dating from 50 to 5 ka BP from the region of the Swiss and Swabian Jura is the first regional study of Pleistocene to early Holocene horse population development. The investigated time frame includes the LGM as a major environmental change, which was followed by open steppe and later afforestation after the temperature rise c. 14.7 ka BP, leading to a densely forested landscape which was in turn partially re-opened for humans' agricultural demands. The archaeological context of the horse remains studied here mirror hunter-gatherer colonisation, settlement and hunting strategies in the region. We have investigated all known *E. f. caballus* remains from Pleistocene Switzerland; open-air dry- and wetland sites did not contain specimens with amplifiable mtDNA [29].

In the region of the Swiss and Swabian Jura, we observe only little continuity of horse matrilines through time, particularly in the populations present during (Badegoulian) and directly

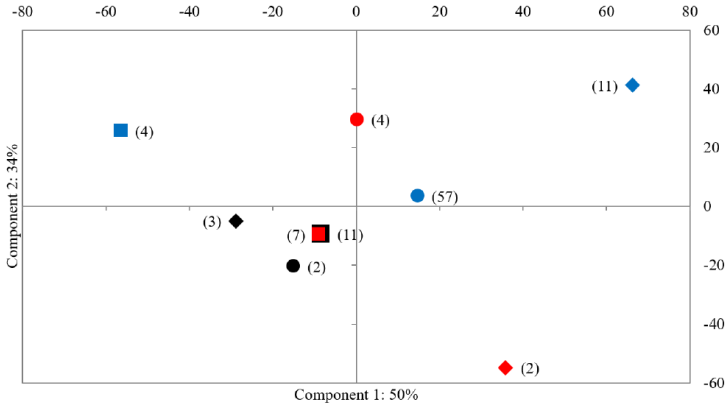


Fig 5. PCA graph based on relative haplogroup frequencies. Asia (black), Ural region (red) and Swiss/Swabian (blue) samples divided in time bins: square = before the LGM, diamond = LGM, circle = after the LGM. Sample numbers are given in parenthesis. The first two components explain 84% of the variation (see S4 Fig for loadings).

https://doi.org/10.1371/journal.pone.0177458.g005

after (Magdalenian) the LGM. Horses were most abundant when the landscape was open and comprised the typical characteristics of steppe biota like herbaceous grassland interspersed with shrub flora. Statistical analyses portend population expansion in the Magdalenian. Despite numerous archaeological sites from the Azilian, the Mesolithic and the Neolithic, horse finds from these horizons are extremely rare [19, 26]. Population fragmentation is indicated by comparatively high nucleotide and low haplotype diversity, and for the Neolithic deme, expansion can be rejected based on a significant raggedness index.

Throughout the investigated time frame, diversity patterns in Eurasia change. The general trend of decreasing nucleotide and haplotype diversity from east to west supports models of an initial population expansion of wild horses in eastern Asia [55]. However, the idea of a panmictic horse population across Eurasia during the last 50 ka (e.g. [1, 11]) has to be challenged, even for pre-LGM times, based on relative haplogroup frequencies. During the LGM, when large parts of the continent were covered by ice or un-inhabitable due to extremely cold and arid conditions [56], populations were isolated and fragmented as partially supported by F_{ST} values. Subsequently, this led to a diversification particularly in the Ural and Swiss/Swabian Jura regions. The finding of a regional population expansion in the Magdalenian contradicts previous studies of horse population development [1, 12] that proposed a rapid decline directly after the LGM.

Wild horses might have gone extinct in the region of present-day Switzerland in the Neolithic, yet due to low numbers of remains in the archaeological record from the Azilian onwards it is challenging to trace this development genetically. It seems likely that climate, and not human activity, was the major driving force behind abundance and diversity of horse populations in this region: the expansion time is contemporaneous with intensified human encroachment of the area after the LGM. However, Neolithic land use for farming and domestic animal husbandry presumably replaced the last remnants of the wild horse population in Switzerland; genetically, this remains to be proven.

Conclusion

In summary, the region of present-day Switzerland was inhabited by discontinuous horse populations and we cannot assume a panmictic deme over the investigated time period of 50 ka. Horse populations mostly replaced each other during and after the LGM, and only little continuity is observable. In the Magdalenian, diversity was highest as the population expanded into the newly accessible landscape. When the landscape transformed from open steppe into more and more densely forested woodland, the population probably shrunk and became fragmented.

Comparing horse matrilineages from Asia, the Ural, and the Swiss and Swabian Jura regions, population-specific developments are detectable. Wild horses possibly never formed a panmictic deme throughout their distribution range, and the LGM led to additional population fragmentation which subsequently persisted.

Besides the methodological challenges due to the discontinuous and unbalanced representation of equid sequences, this paper provides the first comprehensive investigation of wild horse remains from one restricted region. This approach has offered the opportunity to focus on aspects of horse population development that might be overlooked in the global picture by demonstrating specific reaction patterns to changing environmental conditions.

Supporting information

S1 Fig. Density plots of randomisation test (10,000 permutations with replacement) based on nucleotide (left panel) and haplotype (right panel) diversity for datasets 1–3. Unbiased

and thus comparable pairs are framed. A: Dataset 1; B: Dataset 2; C: Dataset 3.
(DOCX)

S2 Fig. Mismatch distribution (observed, bold line, and expected, dashed line) within time bins. X-axis: pairwise differences, y-axis: number of pairs. A: Dataset 1; B: Dataset 2; C: Dataset 3.
(DOCX)

S3 Fig. Density plots of randomization of Eurasian Pleistocene horse sample groups (10 k permutations with replacement) based on nucleotide diversity. Swiss/Swabian samples: dataset 2. Rejections of null hypothesis are framed blue (Swiss LGM vs. Asia BLGM 0.0208; Swiss LGM vs. Swiss PLGM 0.034).
(DOCX)

S4 Fig. Influential haplogroups (loadings) of component 1 (left panel) and 2 (right panel) for PCA graph.
(DOCX)

S1 Table. Details of investigated sites, including site context, main references, location, laboratory and archaeological code, skeletal element, GenBank accession code, and dates [extended from 29].
(DOCX)

S2 Table. Parameters for weighting of nucleotide positions for Median Joining Network analysis based on 97 Pleistocene horse mitochondrial d-loop sequences.
(DOCX)

S3 Table. Details of haplogroups detected in Pleistocene horses from the Swiss and Swabian Jura region, nomenclature follows [2]. Haplogroup defining nucleotide positions relative to the horse reference mitogenome [1] are shown according to their position. All deviations from the reference sequence are given, with mandatory defining positions in bold and optional nucleotide positions in parenthesis. Note that transitions on nucleotide positions 15,585; 15,604 and 15,650 occur sporadically in all haplogroups; these positions are regarded as hotspots and therefore dismissed.
(DOCX)

S4 Table. Nucleotide and haplotype diversities in horse populations from Switzerland and the Swabian Jura (all datasets).
(DOCX)

S5 Table. F_{ST} values of pairwise populations (all datasets). Lower triangle: F_{ST} values, upper triangle: *p* values. Comparable populations are boxed, significant F_{ST} values are in bold.
(DOCX)

S6 Table. Tajima's *D*, Fu's F_S, sum of squared deviances (SSD) and Harpending's raggedness index results for horse populations from Switzerland and the Swabian Jura (all datasets). Significant results are in bold. NaN = not a number because only one haplotype was present.
(DOCX)

S7 Table. Sequences of published Pleistocene horses from Eurasia. Sequences marked with an "a" are part of draft full genomes which are obtainable as SRA-Illumina runs on GenBank.
(DOCX)

S8 Table. Nucleotide and haplotype diversity of Eurasian Pleistocene horses based on pairwise deletion of missing nucleotides.
(DOCX)

S9 Table. F_{ST} values of Eurasian Pleistocene horses. Lower triangle: F_{ST} values, upper triangle: *p* values. Comparable populations are boxed, significant F_{ST} values are in bold. Swiss/Swabian samples: dataset 2.
(DOCX)

Acknowledgments

Samples were kindly provided by Kantonsarchäologie Aargau (Mumpf), Archäologische Bodenforschung Basel-Stadt (Riehen-Ausserberg), Naturhistorisches Museum Basel (Allschwil-Ziegelei, Birseck-Ermitage, Münchenstein-Steinbruch, Schalberghöhle), Archäologie und Kantonsmuseum Basel-Landschaft (Brügglihöhle, Kohlerhöhle), Archäologischer Dienst Bern (Abri Neumühle, Twann-Bahnhof), Université de Neuchâtel (Hauterive-Champréveyres, Monruz), Kontansarchäologie Schaffhausen (Kesslerloch, Schweizersbild), Kantonsarchäologie Solothurn (Käsloch, Rislisberg) and by J. Sedlmeier. The authors thank L. Orlando and M. Muschick for help with statistical analysis. This study was funded by Swiss National Science Foundation (K-31K1_120528/1), Freiwillige Akademische Gesellschaft Basel, and Nikolaus and Bertha Burckhardt-Bürgin-Foundation.

Author Contributions

Conceptualization: JS AS.

Data curation: JE.

Formal analysis: JE MH AS.

Funding acquisition: JS AS.

Investigation: JE.

Methodology: JE MH AS.

Project administration: JS AS.

Resources: JS AS.

Supervision: MH JS AS.

Visualization: JE.

Writing – original draft: JE.

Writing – review & editing: JE MH JS AS.

References

1. Lorenzen ED, Nogues-Bravo D, Orlando L, Weinstock J, Binladen J, Marske KA, et al. Species-specific responses of Late Quaternary megafauna to climate and humans. Nature. 2011; 479(7373):359–64. https://doi.org/10.1038/nature10574 PMID: 22048313
2. Crees JJ, Carbone C, Sommer RS, Benecke N, Turvey ST. Millennial-scale faunal record reveals differential resilience of European large mammals to human impacts across the Holocene. P Roy Soc Lond B Bio. 2016; 283(1827).
3. McCain CM, King SB. Body sizes and activity times mediate mammalian responses to climate change. Global Change Biol. 2014.

4. Campos PF, Willerslev E, Sher A, Orlando L, Axelsson E, Tikhonov A, et al. Ancient DNA analyses exclude humans as the driving force behind late Pleistocene musk ox (*Ovibos moschatus*) population dynamics. PNAS. 2010; 107(12):5675–80. https://doi.org/10.1073/pnas.0907189107 PMID: 20212118
5. Barnosky AD, Koch PL, Feranec RS, Wing SL, Shabel AB. Assessing the causes of late Pleistocene extinctions on the continents. Science. 2004; 306(5693):70–5. https://doi.org/10.1126/science.1101476 PMID: 15459379
6. Koch PL, Barnosky AD. Late quaternary extinctions: State of the debate. Annu Rev Ecol Evol S. 2006; 37:215–50.
7. Hofreiter M, Stewart J. Ecological change, range fluctuations and population dynamics during the Pleistocene. Curr Biol. 2009; 19(14):R584–R94. https://doi.org/10.1016/j.cub.2009.06.030 PMID: 19640497
8. Stuart AJ. Late Quaternary megafaunal extinctions on the continents: a short review. Geol J. 2015; 50(3):338–63.
9. Vila C, Leonard JA, Gotherstrom A, Marklund S, Sandberg K, Liden K, et al. Widespread origins of domestic horse lineages. Science. 2001; 291(5503):474–7. https://doi.org/10.1126/science.291.5503.474 PMID: 11161199
10. Weinstock J, Willerslev E, Sher A, Tong W, Ho SY, Rubenstein D, et al. Evolution, systematics, and phylogeography of Pleistocene horses in the new world: a molecular perspective. PLoS Biol. 2005; 3(8):e241. https://doi.org/10.1371/journal.pbio.0030241 PMID: 15974804
11. Cieslak M, Pruvost M, Benecke N, Hofreiter M, Morales A, Reissmann M, et al. Origin and history of mitochondrial DNA lineages in domestic horses. PLoS ONE. 2010; 5(12):e15311. https://doi.org/10.1371/journal.pone.0015311 PMID: 21187961
12. Orlando L, Ginolhac A, Zhang G, Froese D, Albrechtsen A, Stiller M, et al. Recalibrating *Equus* evolution using the genome sequence of an early Middle Pleistocene horse. Nature. 2013; 499(7456):74–8. https://doi.org/10.1038/nature12323 PMID: 23803765
13. Buoncristiani J-F, Campy M. The palaeogeography of the last two glacial episodes in France: the Alps and Jura. In: Ehlers J, Gibbard PL, editors. Quaternary Glaciations Extent and Chronology Part I: Europe. Amsterdam: Elsevier; 2004. p. 101–10.
14. Ivy-Ochs S, Kerschner H, Reuther A, Maisch M, Sailer R, Schaefer J, et al. The timing of glacier advances in the northern European Alps based on surface exposure dating with cosmogenic 10Be, 26Al, 36Cl, and 21Ne. Geol S Am S. 2006; 415:43–60.
15. Preusser F, Graf HR, Keller O, Krayss E, Schlüchter C. Quaternary glaciation history of northern Switzerland. Quatern Sci J. 2011; 60(2–3):282–305.
16. Ivy-Ochs S, Schafer J, Kubik PW, Synal HA, Schluchter C. Timing of deglaciation on the northern Alpine foreland (Switzerland). Eclogae Geol Helv. 2004; 97(1):47–55.
17. Jäger H, Achermann M, Waroszewski J, Kabała C, Malkiewicz M, Gärtner H, et al. Pre-alpine mire sediments as a mirror of erosion, soil formation and landscape evolution during the last 45 ka. CATENA. 2015; 128(0):63–79.
18. Ivy-Ochs S, Kerschner H, Reuther A, Preusser F, Heine K, Maisch M, et al. Chronology of the last glacial cycle in the European Alps. J Quatern Sci. 2008; 23(6–7):559–73.
19. Nielsen EH. Paläolithikum und Mesolithikum in der Zentralschweiz. Mensch und Umwelt zwischen 17 000 und 5 500 v. Chr. Luzern: Archäologische Schriften; 2009.
20. Leesch D, Müller W, Nielsen E, Bullinger J. The Magdalenian in Switzerland: Re-colonization of a newly accessible landscape. Quatern Int. 2012; 272(0):191–208.
21. Cupillard C, Magny M, Bocherens H, Bridault A, Bégeot C, Bichet V, et al. Changes in ecosystems, climate and societies in the Jura Mountains between 40 and 8 ka cal BP. Quatern Int. 2014(0).
22. Ammann B, von Grafenstein U, van Raden UJ. Biotic responses to rapid warming about 14,685yr BP: Introduction to a case study at Gerzensee (Switzerland). Palaeogeogr, Palaeoclimatol, Palaeoecol. 2013; 391(0):3–12.
23. Ammann B, van Leeuwen JFN, van der Knaap WO, Lischke H, Heiri O, Tinner W. Vegetation responses to rapid warming and to minor climatic fluctuations during the Late-Glacial Interstadial (GI-1) at Gerzensee (Switzerland). Palaeogeogr, Palaeoclimatol, Palaeoecol. 2013; 391(0):40–59.
24. Erny-Rodmann C, Gross-Klee E, Haas JN, Jacomet S, Zoller H. Früher "human impact" und Ackerbau im Uebergangsbereich Spätmesolitikum-Frühneolithikum im schweizerischen Mittelland. Jahrbuch der Schweizerischen Gesellschaft für Ur- und Frühgeschichte. 1997; 80:27–56.
25. Schibler J. The economy and environment of the 4th and 3rd millennia BC in the northern Alpine foreland based on studies of animal bones. Environmental Archaeology. 2006; 11(1):49–64.
26. Schibler J, Hüster-Plogmann H. Die neolithische Wildtierfauna und ihr Aussagegehalt betreffend Umwelt und Umweltveränderungen. In: Stöckli WE, Niffeler U, Gross-Klee E, editors. SPM II:

Neolithikum. Die Schweiz vom Paläolithikum bis zum frühen Mittelalter. Basel: Gesellschaft für Ur- und Frühgeschichte der Schweiz; 1995. p. 76–83.

27. Schibler J, Studer J. Haustierhaltung und Jagd während der Bronzezeit der Schweiz. In: Hochuli S, Niffeler U, Rychener J, editors. SPM III: Bronzezeit. Die Schweiz vom Paläolithikum bis zum frühen Mittelalter. Basel: Gesellschaft für Ur- und Frühgeschichte der Schweiz; 1998. p. 171–92.
28. Leesch D. Das späte Jungpaläolithikum. Zeitlicher Rahmen und Fundinventare. In: LeTensorer J-M, Niffeler U, editors. SPM I: Paläolithikum und Mesolithikum. Die Schweiz vom Paläolithikum bis zum frühen Mittelalter. Basel: Gesellschaft für Ur- und Frühgeschichte der Schweiz; 1993. p. 153–64.
29. Elsner J, Schibler J, Hofreiter M, Schlumbaum A. Burial condition is the most important factor for mtDNA PCR amplification success in Palaeolithic equid remains from the Alpine foreland. Archeol Anthropol Sci. 2014; 7(4):505–15.
30. CalPal-2007 online [Internet]. 2012. http://www.calpal-online.de.
31. Shapiro B, Hofreiter M, editors. Ancient DNA—methods and protocols. New York: Springer; 2012.
32. Xu X, Arnason U. The complete mitochondrial DNA sequence of the horse, *Equus caballus*: extensive heteroplasmy of the control region. Gene. 1994; 148(2):357–62. PMID: 7958969
33. Elsner J, Deschler-Erb S, Stopp B, Hofreiter M, Schibler J, Schlumbaum A. Mitochondrial d-loop variation, coat colour and sex identification of Late Iron Age horses in Switzerland. J Archaeol Sci: Rep. 2016; 6:386–96.
34. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acid S. 1999; 41:95–8.
35. Excoffier L, Lischer HE. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour. 2010; 10(3):564–7. https://doi.org/10.1111/j.1755-0998.2010.02847.x PMID: 21565059
36. Tajima F. Evolutionary relationship of DNA sequences in finite populations. Genetics. 1983; 105(2): 437–60. PMID: 6628982
37. Fu YX. Estimating effective population-size or mutation-rate using the frequencies of mutations of various classes in a sample of DNA-sequences. Genetics. 1994; 138(4):1375–86. PMID: 7896116
38. Harpending HC, Sherry ST, Rogers AR, Stoneking M. The genetic structure of ancient human populations. Curr Anthrop. 1993; 34(4):483–96.
39. Harpending HC. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. Hum Biol. 1994; 66(4):591–600. PMID: 8088750
40. Paradis E. pegas: an R package for population genetics with an integrated–modular approach. Bioinformatics. 2010; 26:419–20. https://doi.org/10.1093/bioinformatics/btp696 PMID: 20080509
41. Charif D, Lobry JR. SeqinR 1.0–2: a contributed package to the R project for statistical computing devoted to biological sequences retrieval and analysis. In: Bastolla U, Porto MJ, Roman HE, Vendruscolo M, editors. Structural approaches to sequence evolution: molecules, networks, populations. Biological and Medical Physics, Biomedical Engineering. New York: Springer; 2007. p. 207–32.
42. R Development Core Team RDCT. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2014.
43. Bandelt HJ, Forster P, Rohl A. Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol. 1999; 16(1):37–48. PMID: 10331250
44. Hammer O, Harper DAT, Ryan PD. PAST: PAleontological STatistics software package for education and data analysis. Palaeontol Electron. 2001; 4(1):1–9.
45. Frankham R, Ballou JD, Briscoe DA. Introduction to conservation genetics. Cambridge: Cambridge University Press; 2002. 617 p.
46. Luís C, Bastos-Silveira C, Costa-Ferreira J, Cothran EG, Do Mar Oom M. A lost Sorraia maternal lineage found in the Lusitano horse breed. J Anim Breed Genet. 2006; 123:399–402. https://doi.org/10.1111/j.1439-0388.2006.00612.x PMID: 17177696
47. McGahern A, Bower MAM, Edwards CJ, Brophy PO, Sulimova G, Zakharov I, et al. Evidence for biogeographic pattering of mitochondrial DNA sequences in Eastern horse populations. Anim Genet. 2006; 37(5):494–7. https://doi.org/10.1111/j.1365-2052.2006.01495.x PMID: 16978180
48. Yang Y, Zhu Q, Liu S, Zhao C, Wu C. The origin of Chinese domestic horses revealed with novel mtDNA variants. Animal Science Journal. 2016.
49. Vinther BM, Clausen HB, Johnsen SJ, Rasmussen SO, Andersen KK, Buchardt SL, et al. A synchronized dating of three Greenland ice cores throughout the Holocene. J Geophys Res-Atmos. 2006; 111(D13):D13102.

50. Rasmussen SO, Andersen KK, Svensson AM, Steffensen JP, Vinther BM, Clausen HB, et al. A new Greenland ice core chronology for the last glacial termination. J Geophys Res-Atmos. 2006; 111(D6): D06102.

51. Andersen KK, Svensson A, Johnsen SJ, Rasmussen SO, Bigler M, Röthlisberger R, et al. The Greenland Ice Core Chronology 2005, 15–42ka. Part 1: constructing the time scale. Quatern Sci Rev. 2006; 25(23–24):3246–57.

52. Svensson A, Andersen KK, Bigler M, Clausen HB, Dahl-Jensen D, Davies SM, et al. A 60 000 year Greenland stratigraphic ice core chronology. Clim Past. 2008; 4(1):47–57.

53. Wolff EW, Chappellaz J, Blunier T, Rasmussen SO, Svensson A. Millennial-scale variability during the last glacial: The ice core record. Quatern Sci Rev. 2010; 29(21–22):2828–38.

54. Heiri O, Koinig KA, Spötl C, Barrett S, Brauer A, Drescher-Schneider R, et al. Palaeoclimate records 60–8 ka in the Austrian and Swiss Alps and their forelands. Quatern Sci Rev. 2014(0).

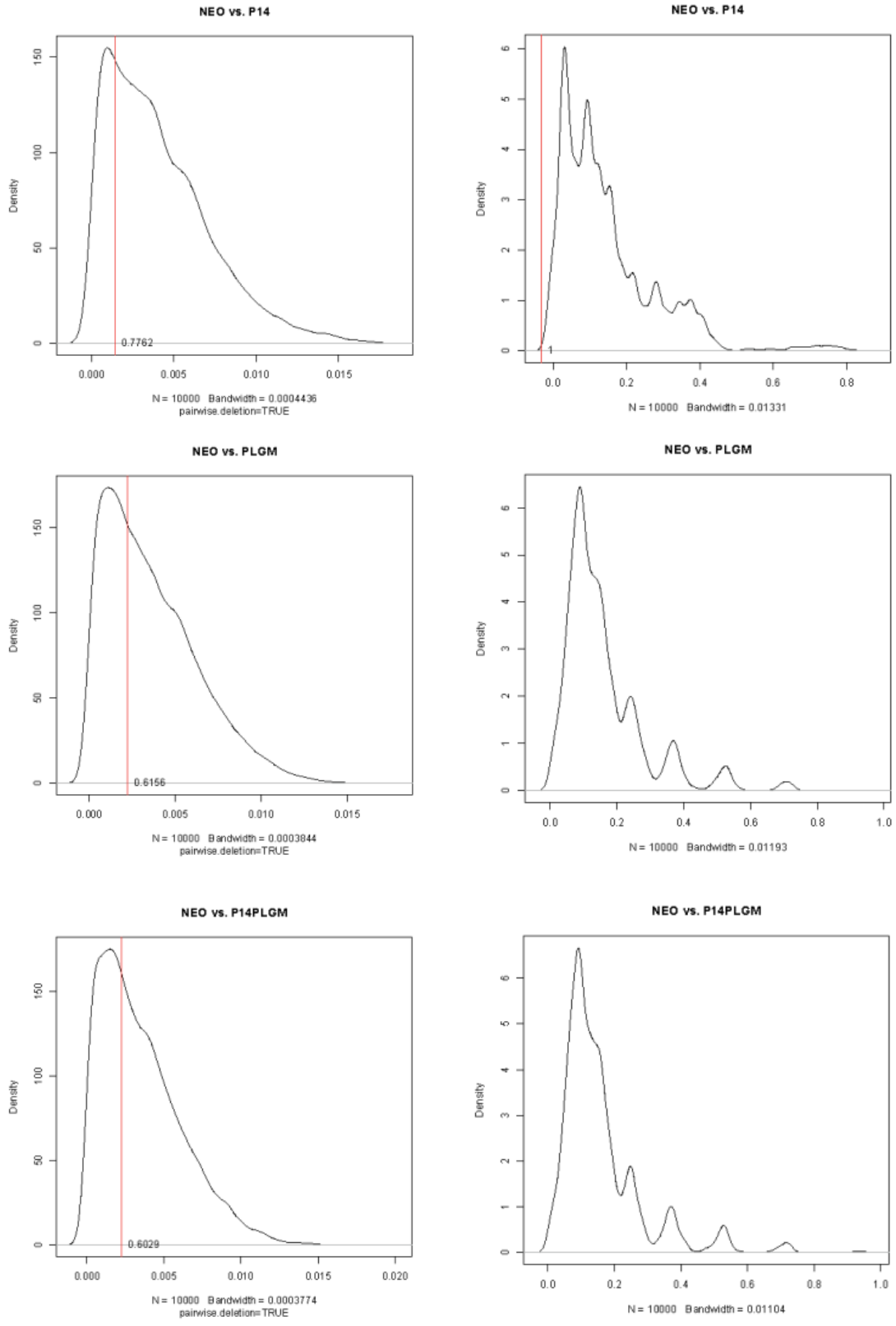
55. Warmuth V, Eriksson A, Bower MA, Barker G, Barrett E, Hanks BK, et al. Reconstructing the origin and spread of horse domestication in the Eurasian steppe. PNAS. 2012; 109(21):8202–6. https://doi.org/10.1073/pnas.1111122109 PMID: 22566639

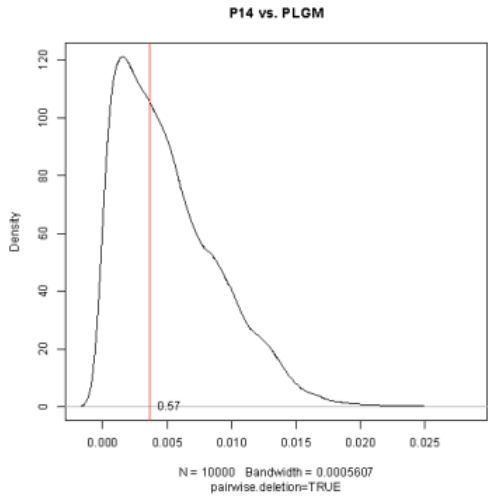
56. Hughes PD, Gibbard PL, Ehlers J. Timing of glaciation during the last glacial cycle: evaluating the concept of a global 'Last Glacial Maximum' (LGM). Earth-Sci Rev. 2013; 125(0):171–98.

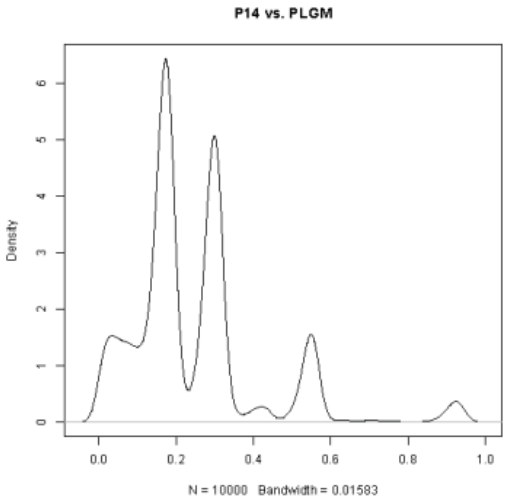
Electronic supplementary material

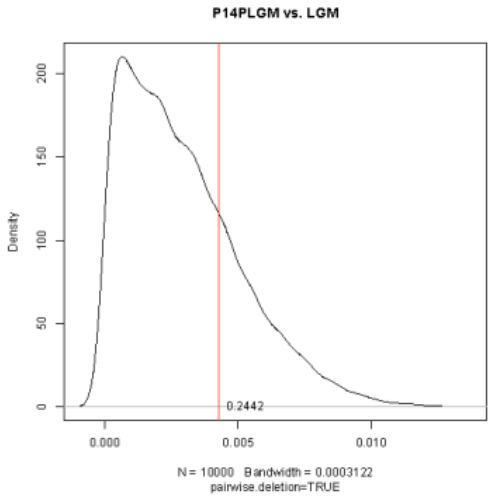
S1 Fig: Density plots of randomisation test (10,000 permutations with replacement) based on nucleotide (left panel) and haplotype (right panel) diversity for datasets 1-3. Unbiased and thus comparable pairs are framed. A: Dataset 1; B: Dataset 2; C: Dataset 3.

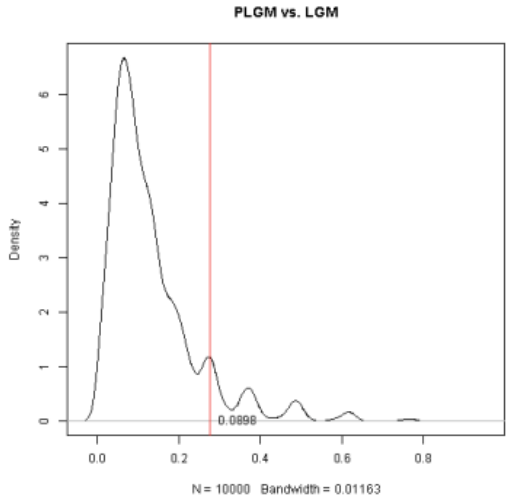
S1 Fig A: Density plots of randomisation test (10,000 permutations with replacement) based on nucleotide (left panel) and haplotype (right panel) diversity. Dataset 1.

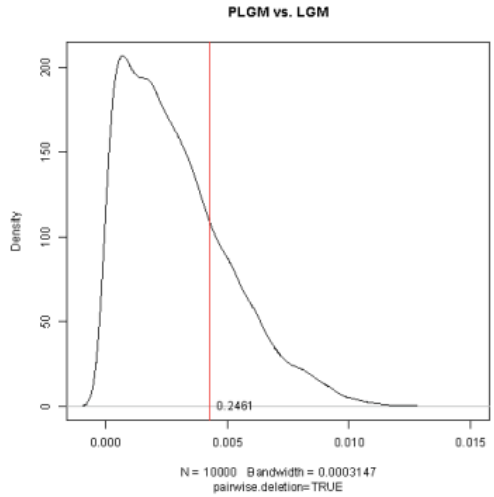


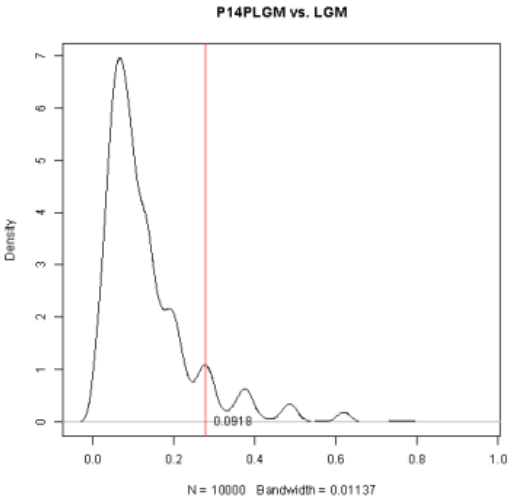

P14 vs. PLGM
Density
0.57
N = 10000 Bandwidth = 0.0005607
pairwise.deletion=TRUE

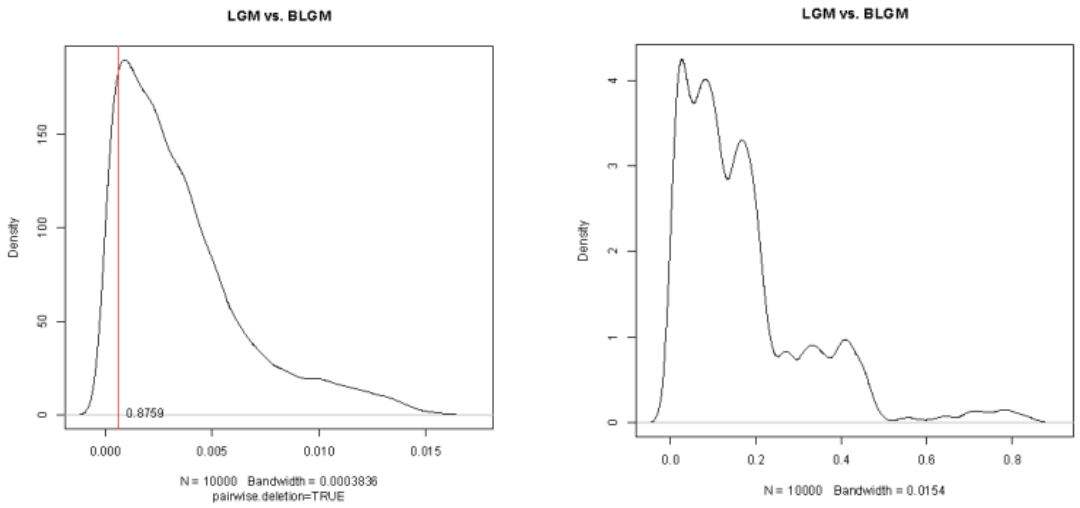

P14 vs. PLGM
Density
N = 10000 Bandwidth = 0.01583


P14PLGM vs. LGM
Density
0.2442
N = 10000 Bandwidth = 0.0003122
pairwise.deletion=TRUE

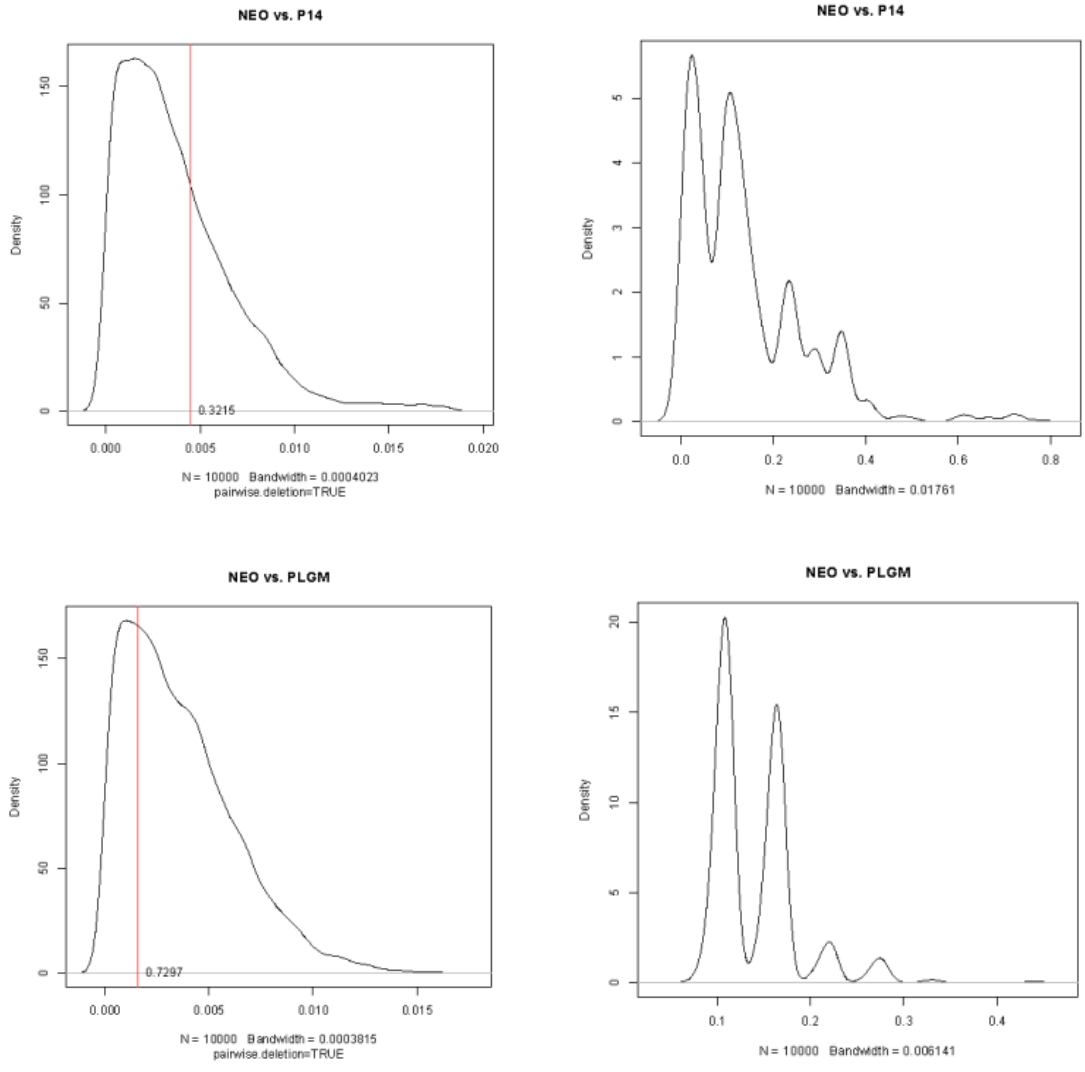

PLGM vs. LGM
Density
0.0898
N = 10000 Bandwidth = 0.01163

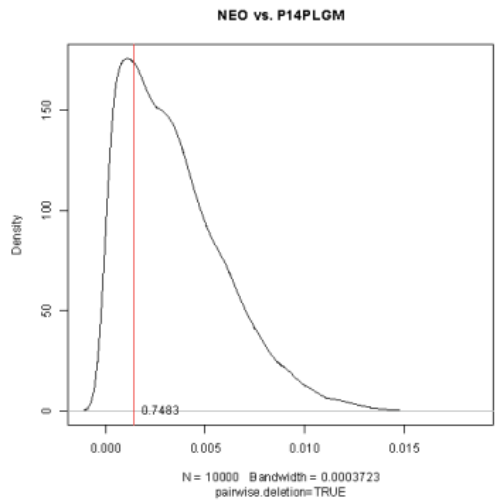

PLGM vs. LGM
Density
0.2461
N = 10000 Bandwidth = 0.0003147
pairwise.deletion=TRUE

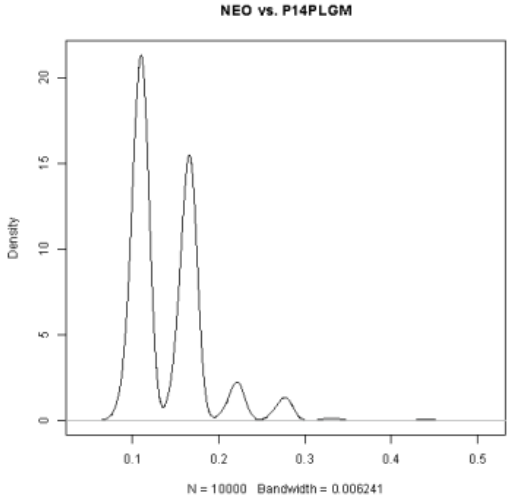

P14PLGM vs. LGM
Density
0.0918
N = 10000 Bandwidth = 0.01137

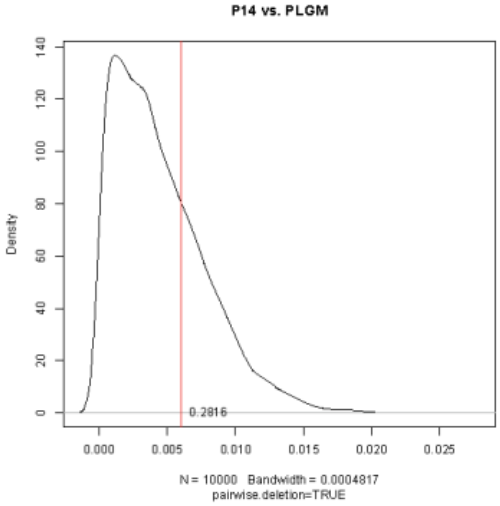


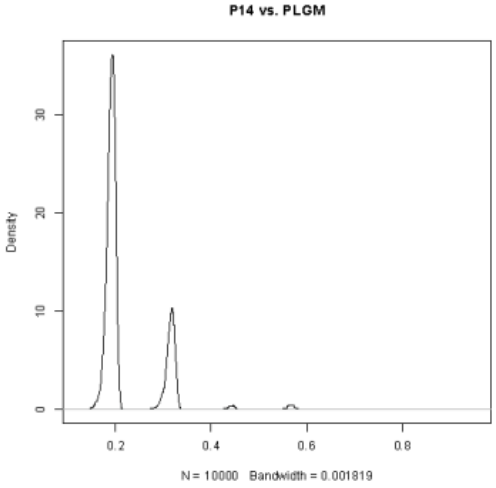
S1 Fig B: Density plots of randomisation test (10,000 permutations with replacement) based on nucleotide (left panel) and haplotype (right panel) diversity. Unbiased and thus comparable pairs are framed blue. Dataset 2.

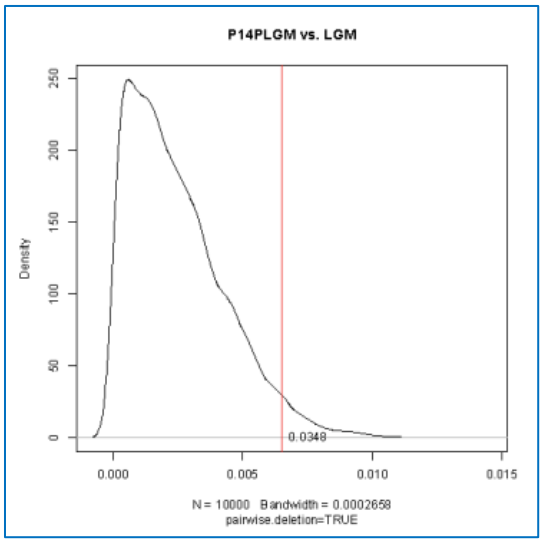


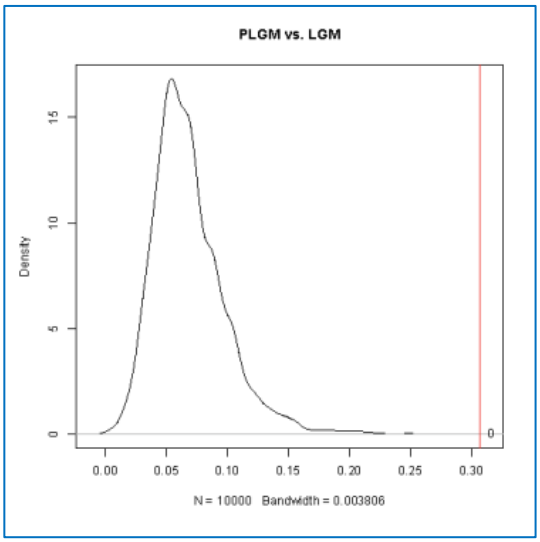

NEO vs. P14PLGM
Density
0.7483
N = 10000 Bandwidth = 0.0003723
pairwise.deletion=TRUE

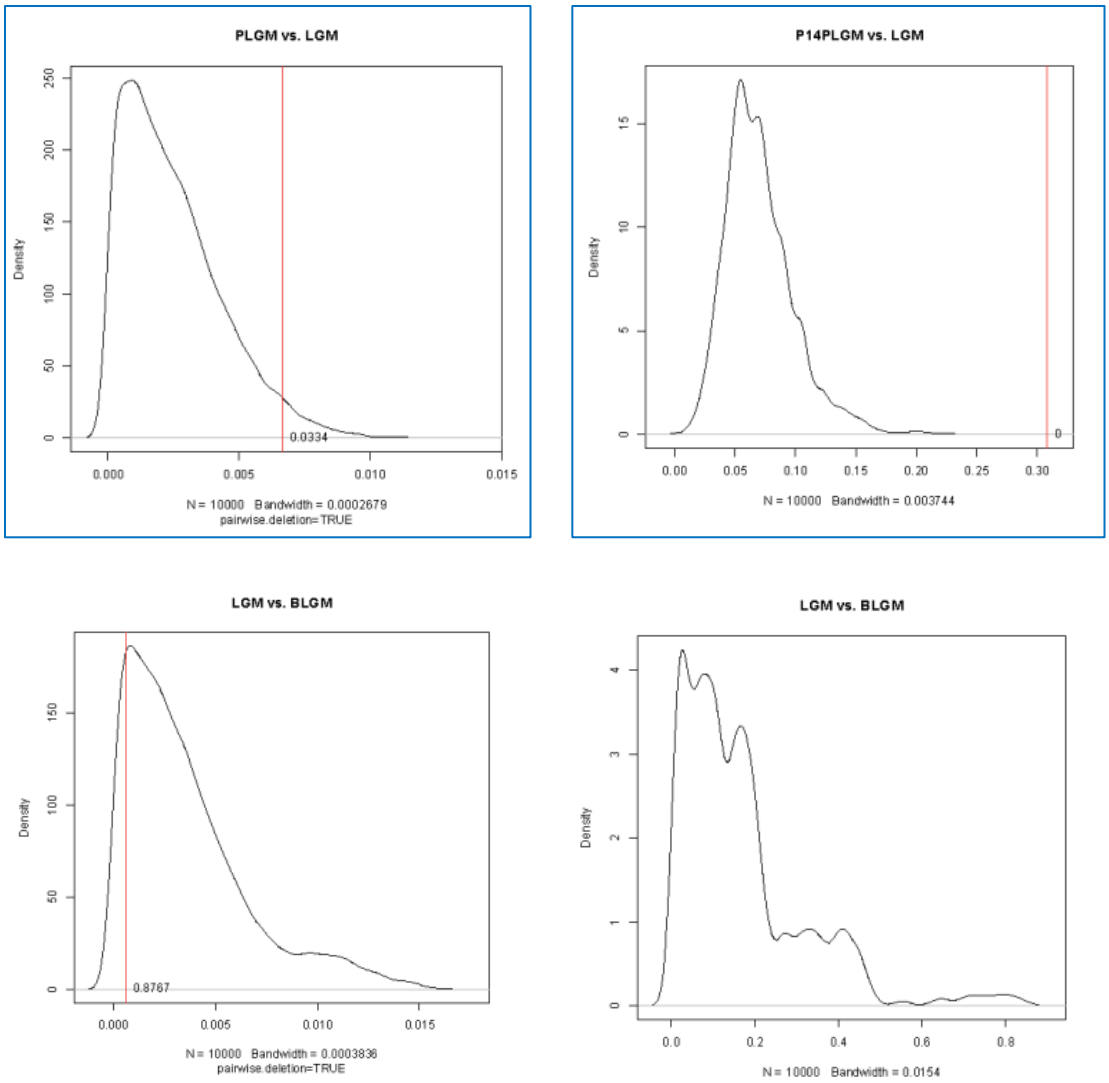

NEO vs. P14PLGM
Density
N = 10000 Bandwidth = 0.006241


P14 vs. PLGM
Density
0.2816
N = 10000 Bandwidth = 0.0004817
pairwise.deletion=TRUE

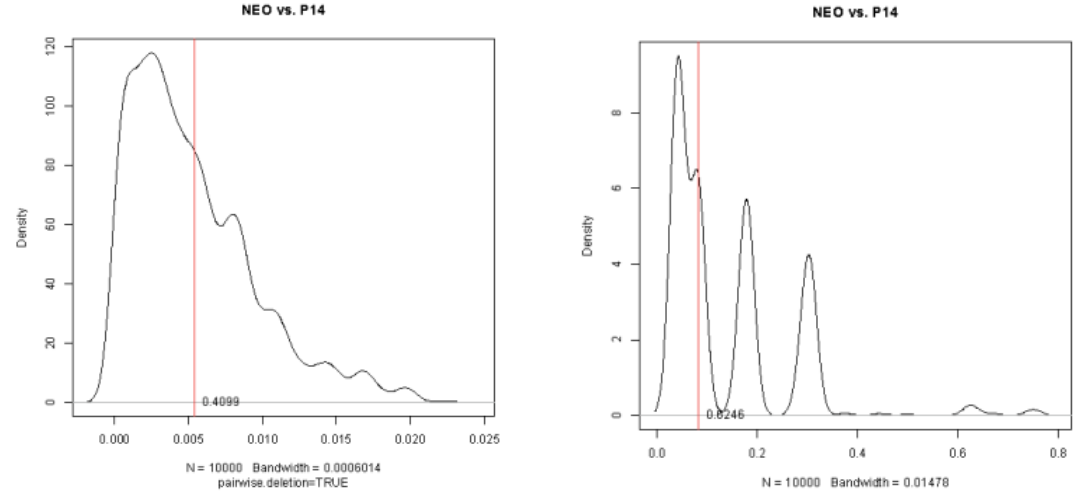

P14 vs. PLGM
Density
N = 10000 Bandwidth = 0.001819

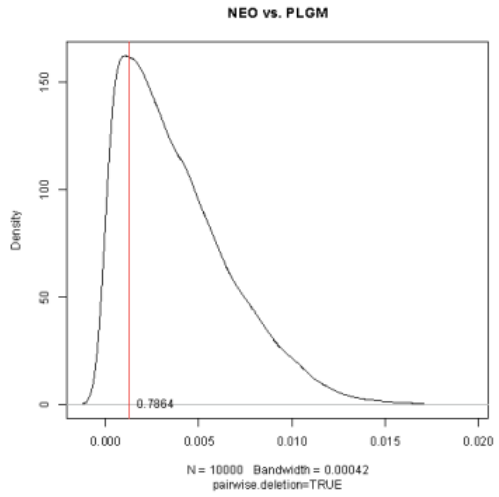

P14PLGM vs. LGM
Density
0.0348
N = 10000 Bandwidth = 0.0002658
pairwise.deletion=TRUE

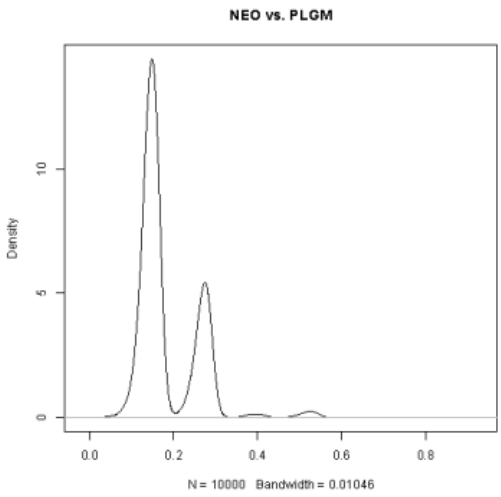

PLGM vs. LGM
Density
0
N = 10000 Bandwidth = 0.003806

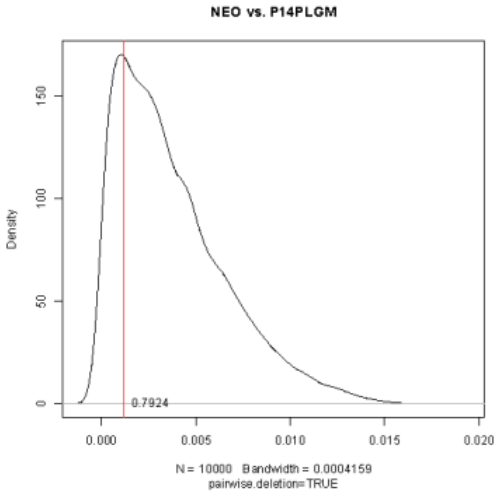


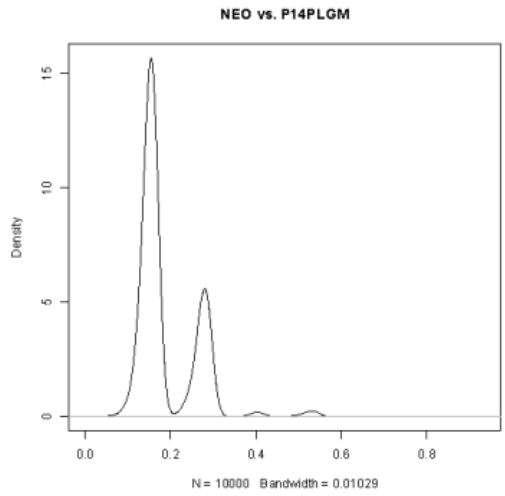
S1 Fig C: Density plots of randomisation test (10,000 permutations with replacement) based on nucleotide (left panel) and haplotype (right panel) diversity. Unbiased and thus comparable pairs are framed blue. Dataset 3.

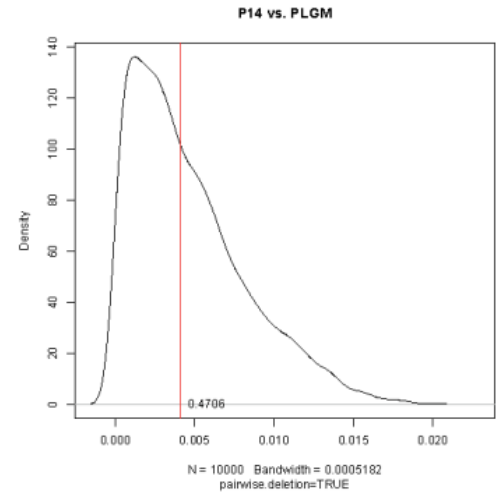


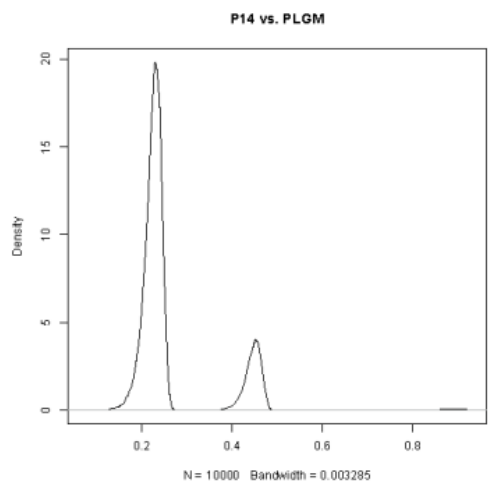

NEO vs. PLGM
Density
0.7864
N = 10000 Bandwidth = 0.00042
pairwise.deletion=TRUE

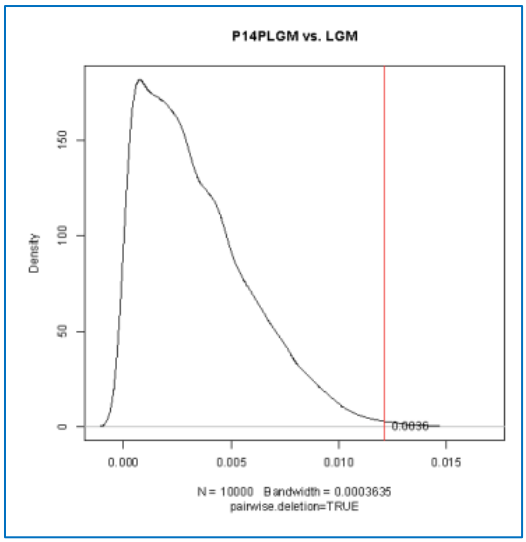

NEO vs. PLGM
Density
N = 10000 Bandwidth = 0.01046

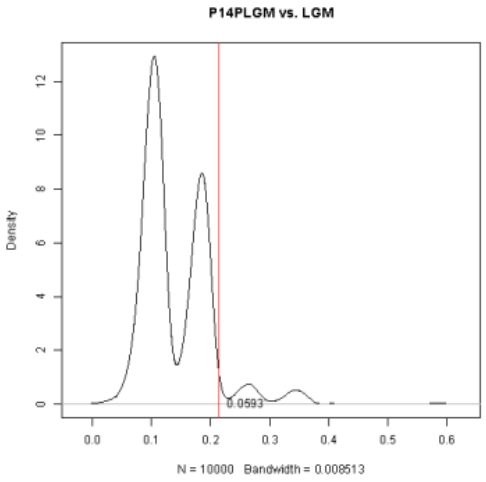

NEO vs. P14PLGM
Density
0.7924
N = 10000 Bandwidth = 0.0004159
pairwise.deletion=TRUE

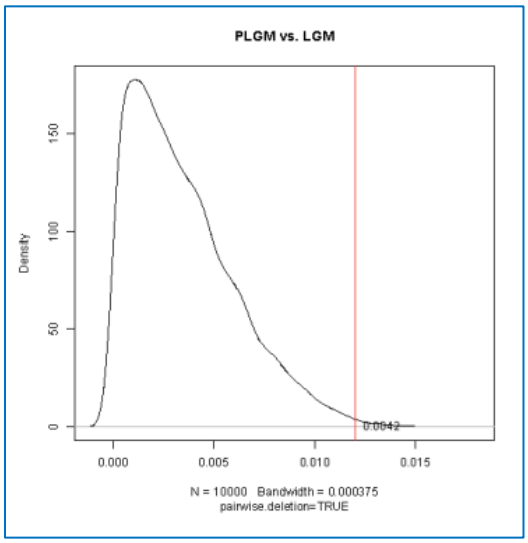

NEO vs. P14PLGM
Density
N = 10000 Bandwidth = 0.01029

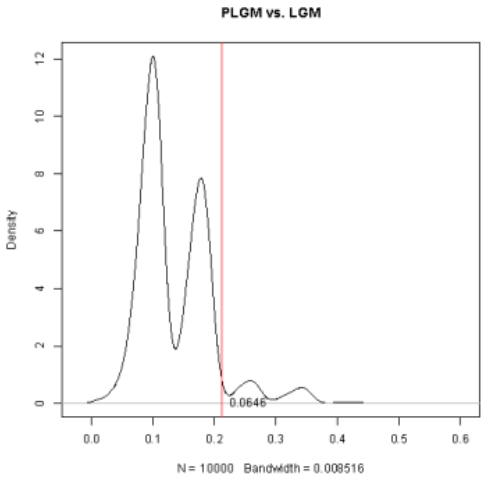

P14 vs. PLGM
Density
0.4706
N = 10000 Bandwidth = 0.0005182
pairwise.deletion=TRUE

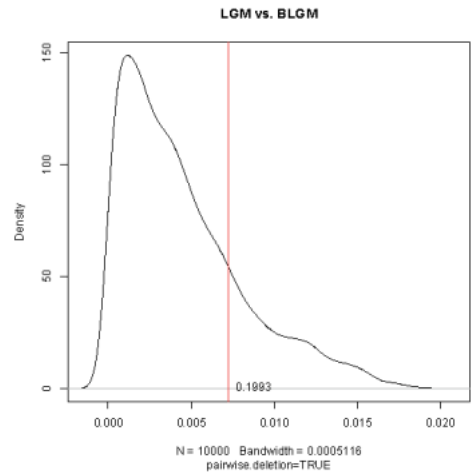

P14 vs. PLGM
Density
N = 10000 Bandwidth = 0.003285

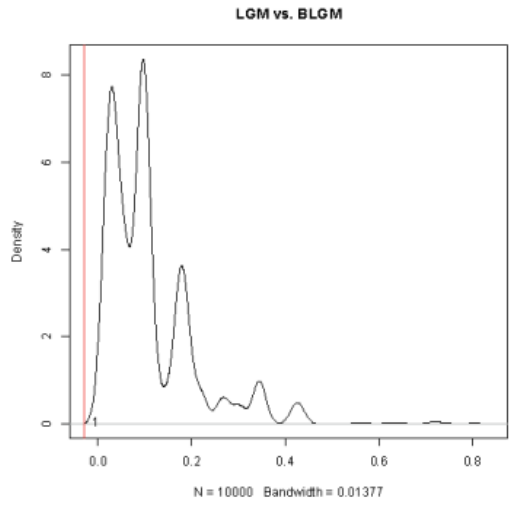

P14PLGM vs. LGM
Density
0.0036
N = 10000 Bandwidth = 0.0003635
pairwise.deletion=TRUE


P14PLGM vs. LGM
Density
0.0593
N = 10000 Bandwidth = 0.008513


PLGM vs. LGM
Density
0.0042
N = 10000 Bandwidth = 0.000375
pairwise.deletion=TRUE

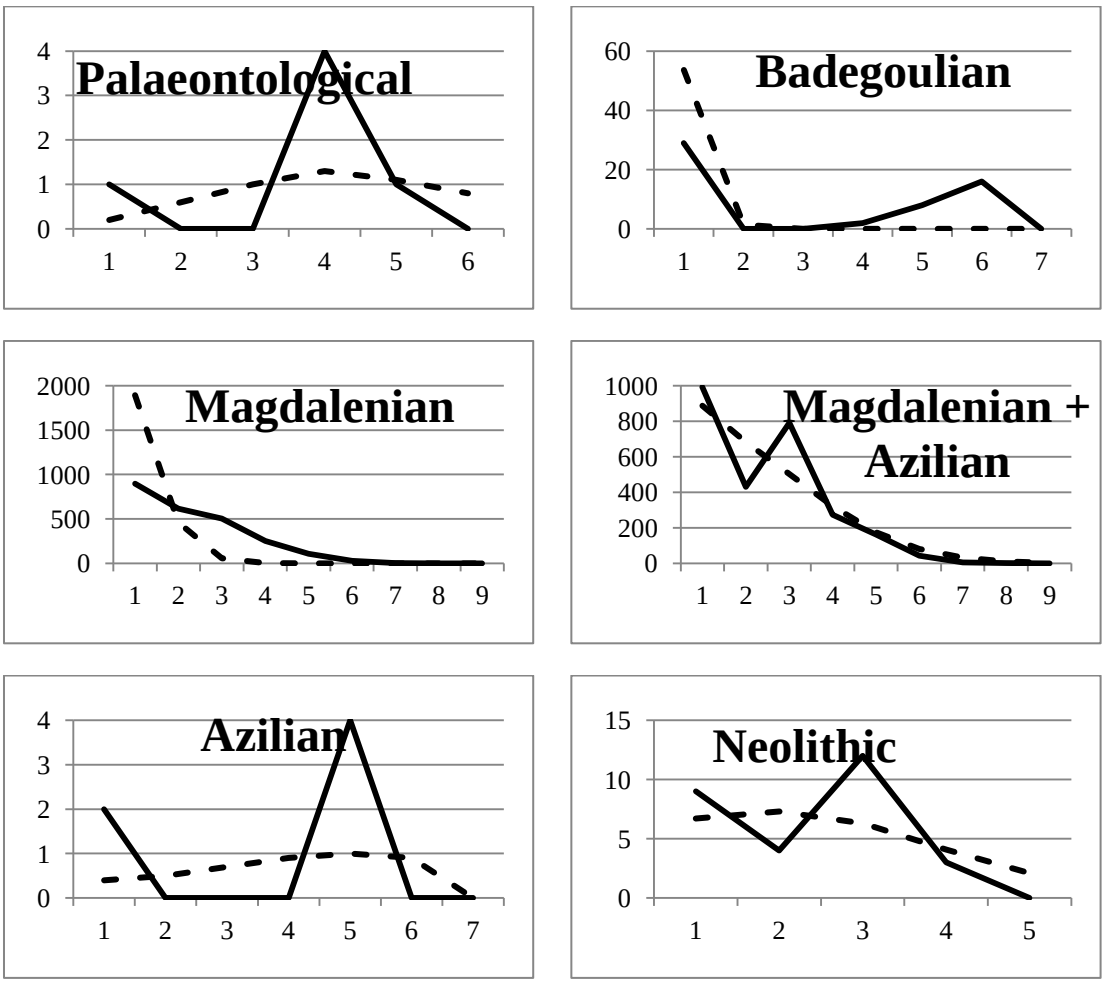

PLGM vs. LGM
Density
0.0646
N = 10000 Bandwidth = 0.008516


LGM vs. BLGM
Density
0.1993
N = 10000 Bandwidth = 0.0005116
pairwise.deletion=TRUE

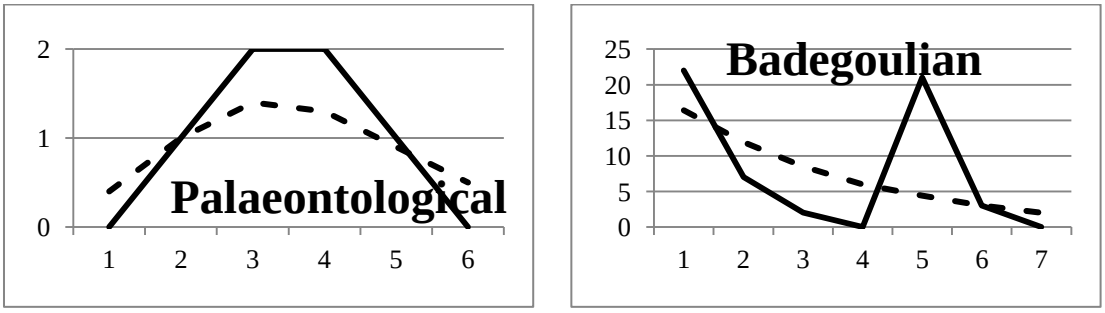

LGM vs. BLGM
Density
1
N = 10000 Bandwidth = 0.01377

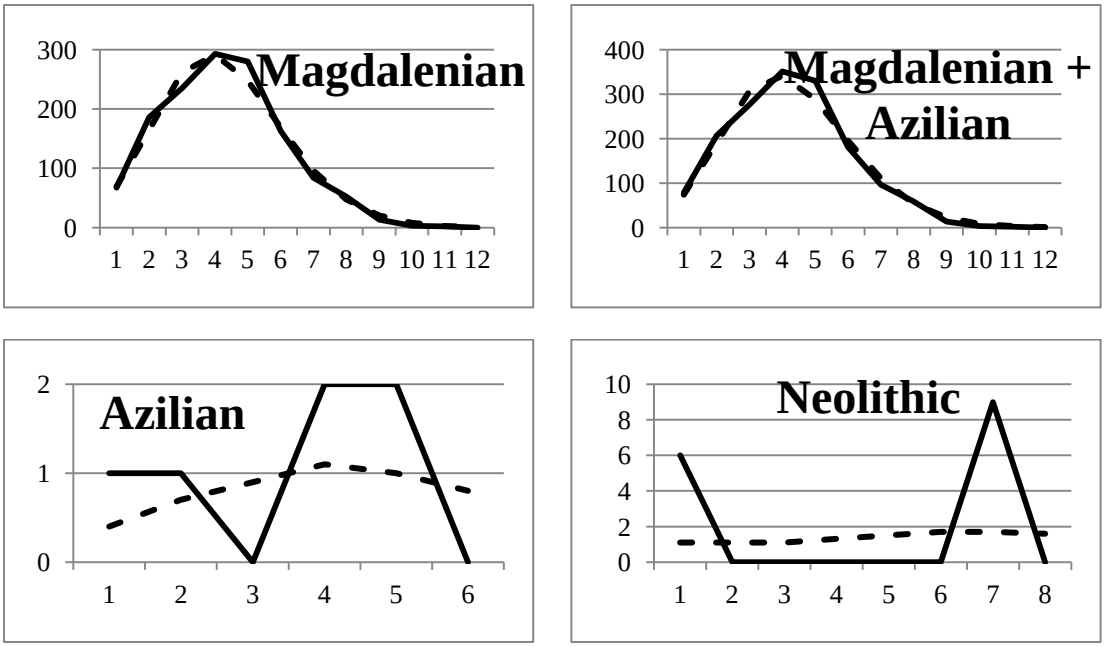
S2 Fig: Mismatch distribution (observed, bold line, and expected, dashed line) within time bins. X-axis: pairwise differences, y-axis: number of pairs. A: Dataset 1; B: Dataset 2; C: Dataset 3.

S2 Fig A: Mismatch distribution (observed, bold line, and expected, dashed line) within time bins. X-axis: paiwise differences, y-axis: number of pairs. Dataset 1.

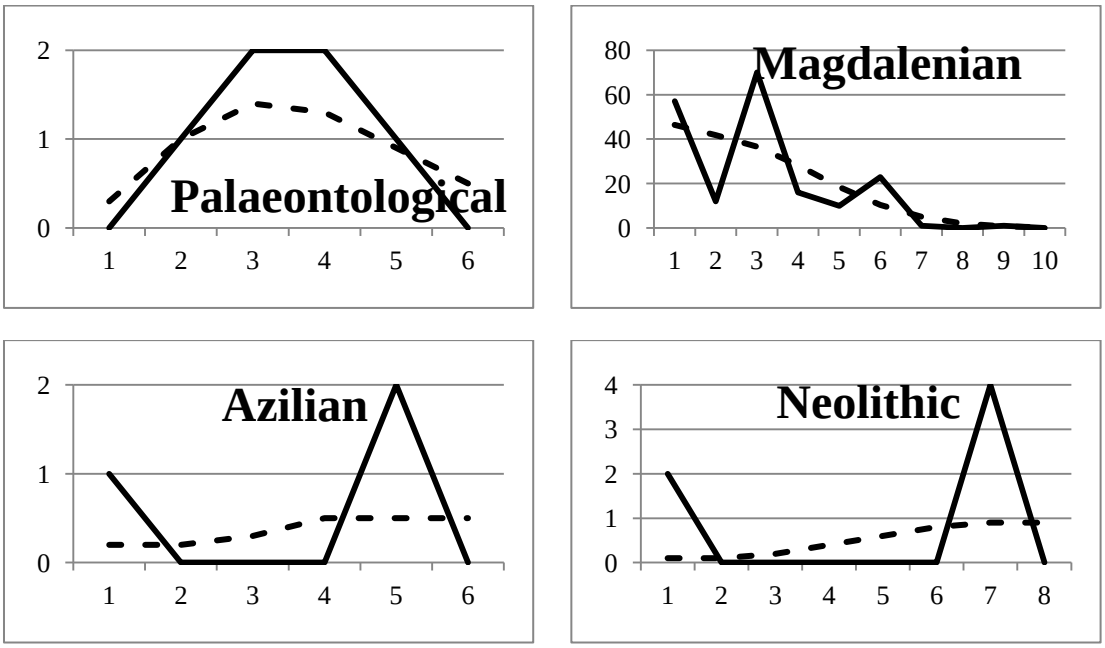


S2 Fig B: Mismatch distribution (observed, bold line, and expected, dashed line) within time bins. X-axis: paiwise differences, y-axis: number of pairs. Dataset 2.

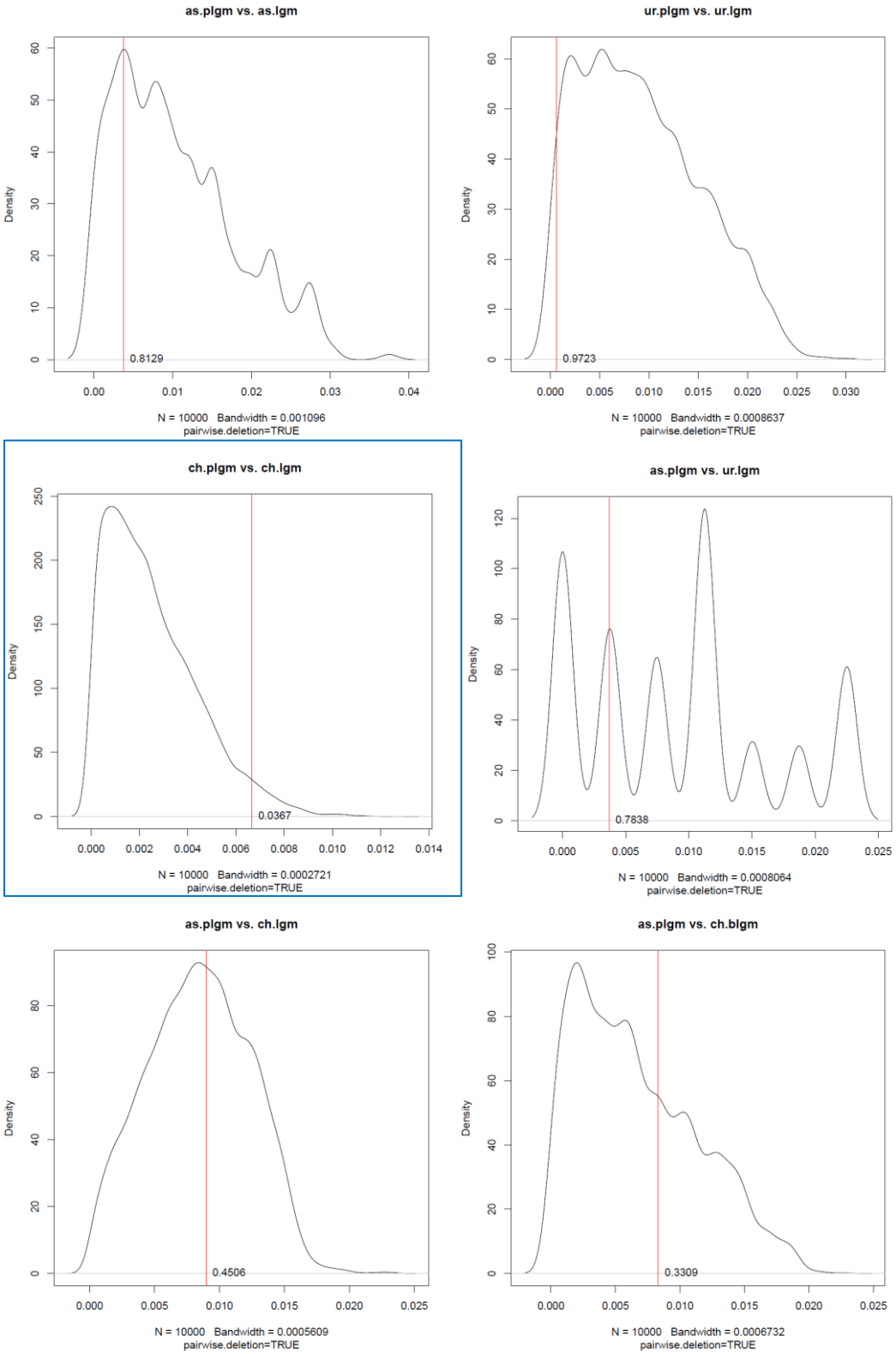


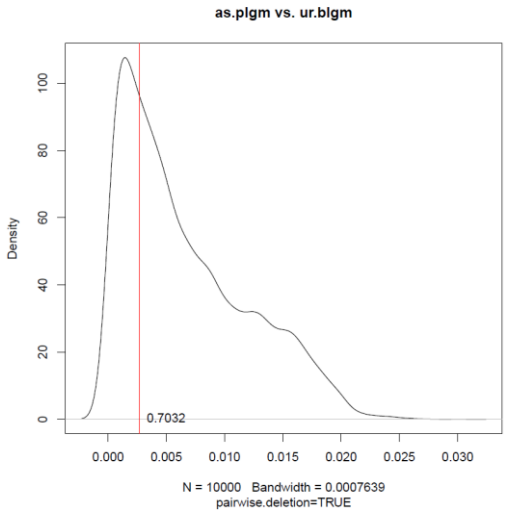


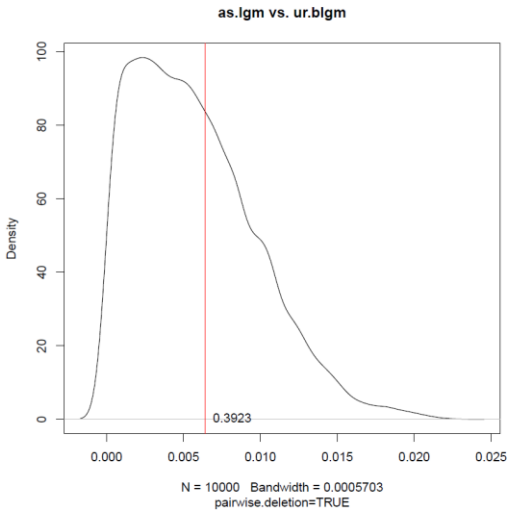
S2 Fig C: Mismatch distribution (observed, bold line, and expected, dashed line) within time bins. X-axis: paiwise differences, y-axis: number of pairs. Dataset 3.

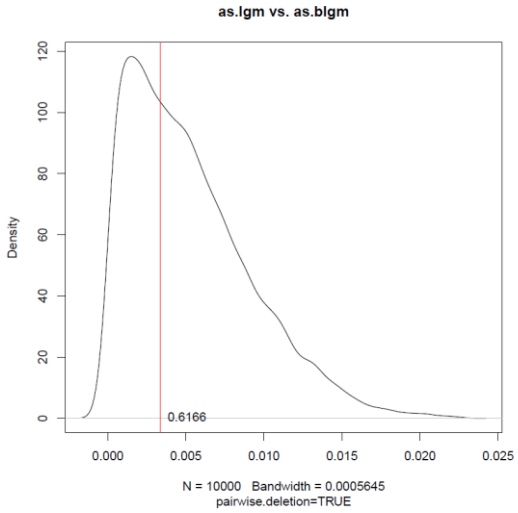


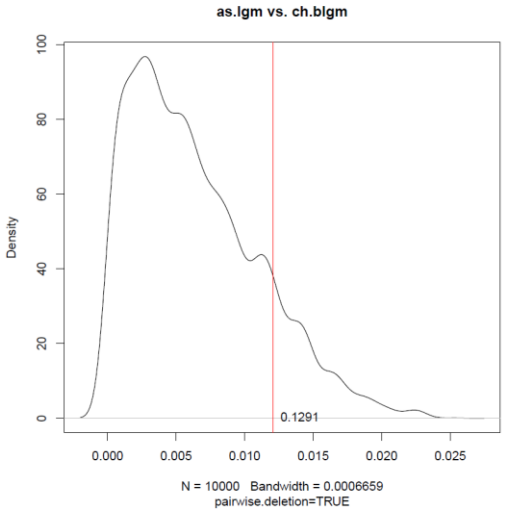
S3 Fig: Density plots of randomization of Eurasian Pleistocene horse sample groups (10 k permutations with replacement) based on nucleotide diversity. Swiss/Swabian samples: dataset 2. Rejections of null hypothesis are framed blue (Swiss LGM vs. Asia BLGM 0.0208; Swiss LGM vs. Swiss PLGM 0.034).

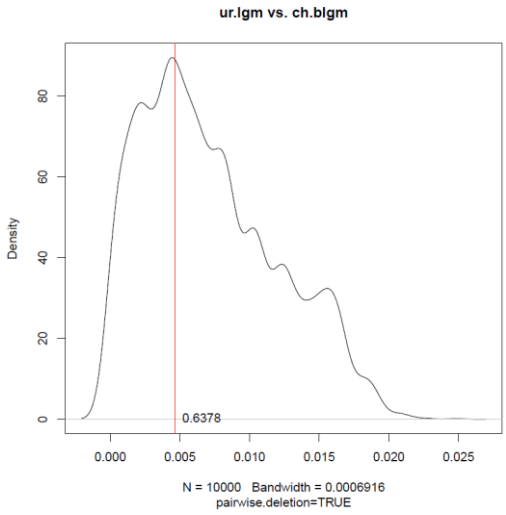


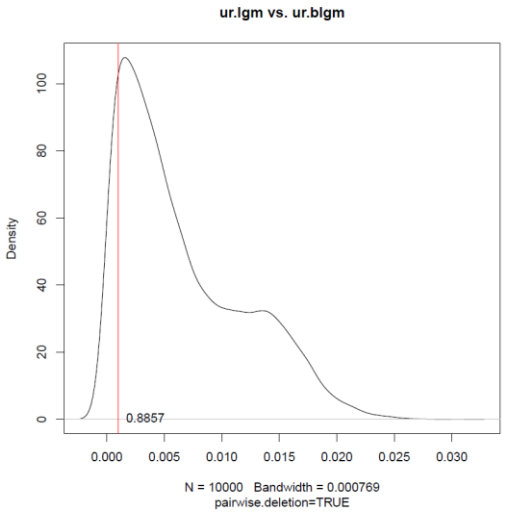

as.plgm vs. ur.blgm
Density
0.7032
N = 10000 Bandwidth = 0.0007639
pairwise.deletion=TRUE

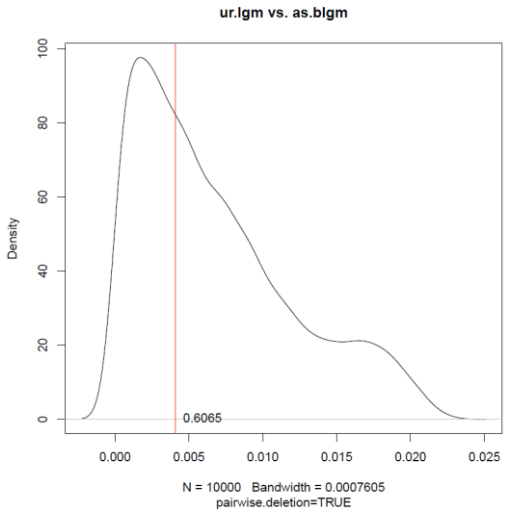

as.lgm vs. ur.blgm
Density
0.3923
N = 10000 Bandwidth = 0.0005703
pairwise.deletion=TRUE

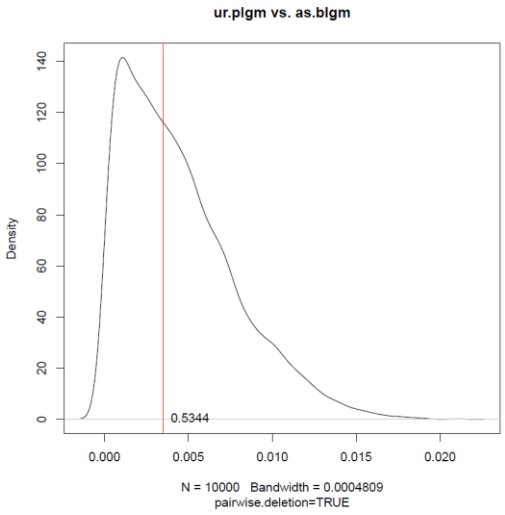

as.lgm vs. as.blgm
Density
0.6166
N = 10000 Bandwidth = 0.0005645
pairwise.deletion=TRUE

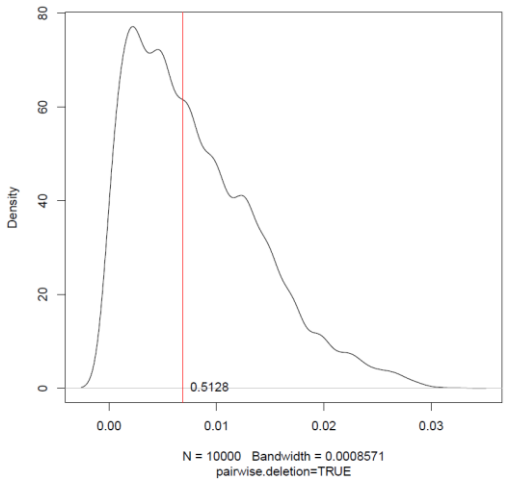

as.lgm vs. ch.blgm
Density
0.1291
N = 10000 Bandwidth = 0.0006659
pairwise.deletion=TRUE

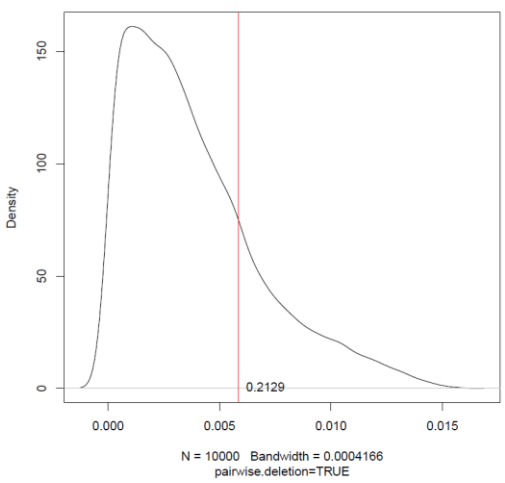

ur.lgm vs. ch.blgm
Density
0.6378
N = 10000 Bandwidth = 0.0006916
pairwise.deletion=TRUE

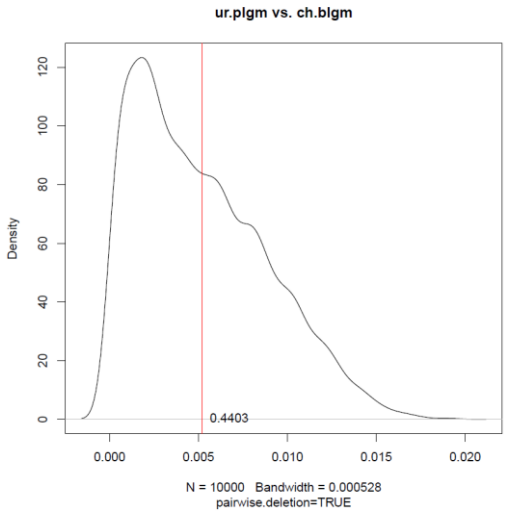

ur.lgm vs. ur.blgm
Density
0.8857
N = 10000 Bandwidth = 0.000769
pairwise.deletion=TRUE

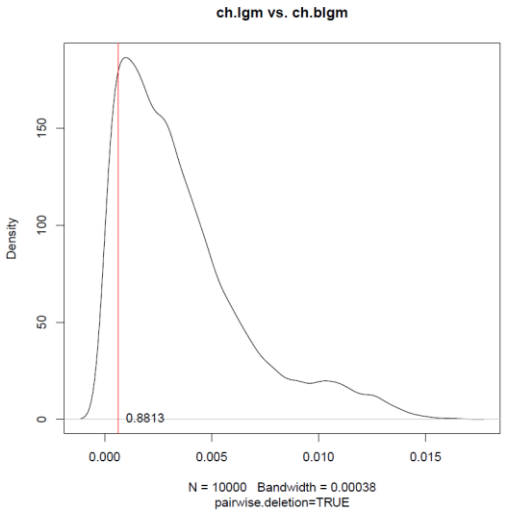

ur.lgm vs. as.blgm
Density
0.6065
N = 10000 Bandwidth = 0.0007605
pairwise.deletion=TRUE

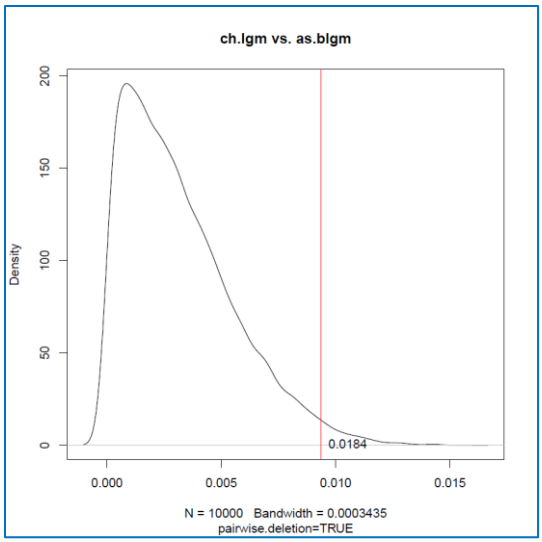

ur.plgm vs. as.blgm
Density
0.5344
N = 10000 Bandwidth = 0.0004809
pairwise.deletion=TRUE

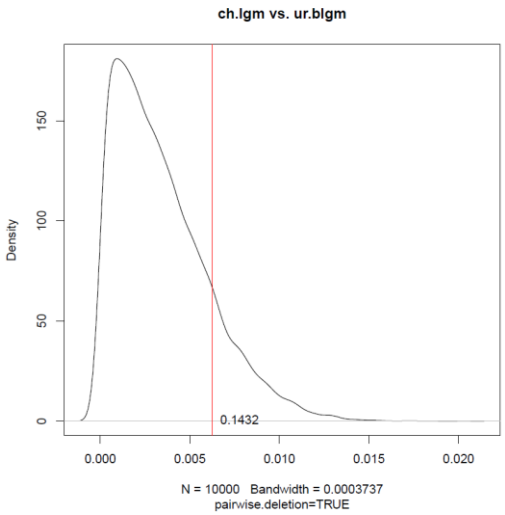

ur.plgm vs. as.lgm
Density
0.5128
N = 10000 Bandwidth = 0.0008571
pairwise.deletion=TRUE

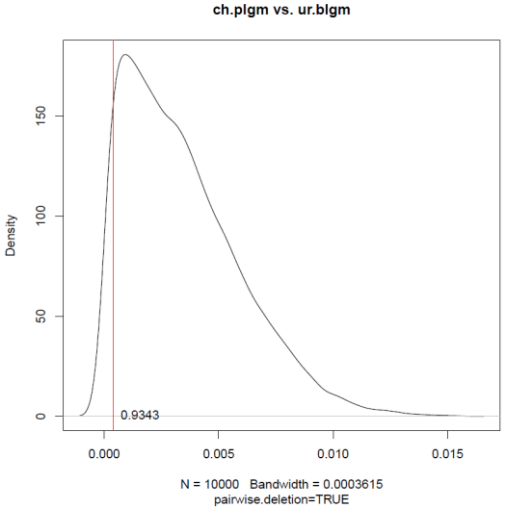

ur.plgm vs. ch.lgm
Density
0.2129
N = 10000 Bandwidth = 0.0004166
pairwise.deletion=TRUE

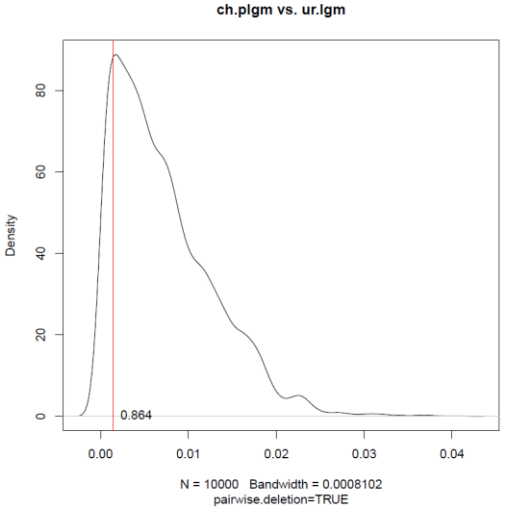

ur.plgm vs. ch.blgm
Density
0.4403
N = 10000 Bandwidth = 0.000528
pairwise.deletion=TRUE

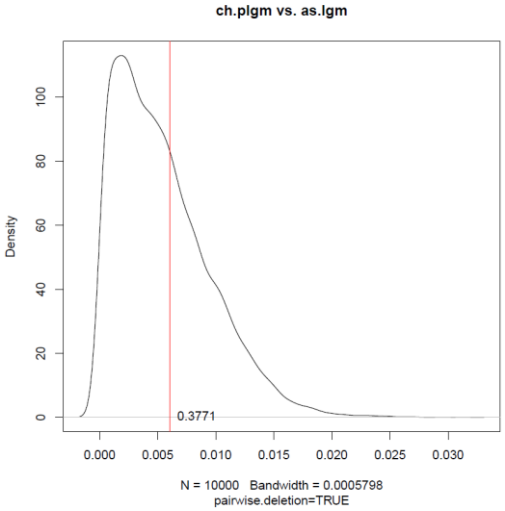

ch.lgm vs. ch.blgm
Density
0.8813
N = 10000 Bandwidth = 0.00038
pairwise.deletion=TRUE

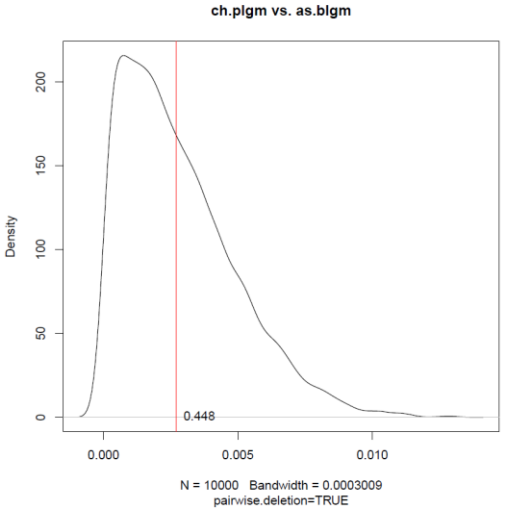

ch.lgm vs. as.blgm
Density
0.0184
N = 10000 Bandwidth = 0.0003435
pairwise.deletion=TRUE

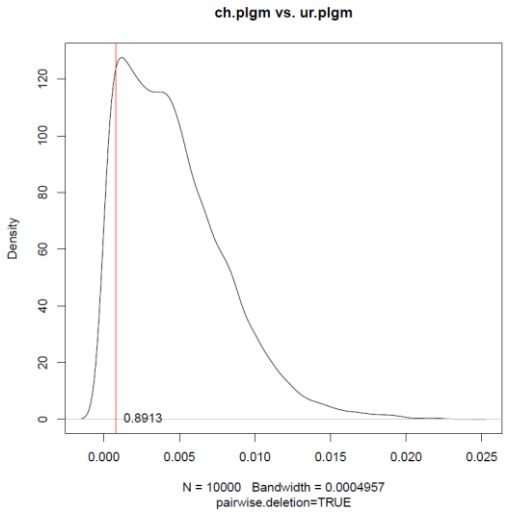

ch.lgm vs. ur.blgm
Density
0.1432
N = 10000 Bandwidth = 0.0003737
pairwise.deletion=TRUE

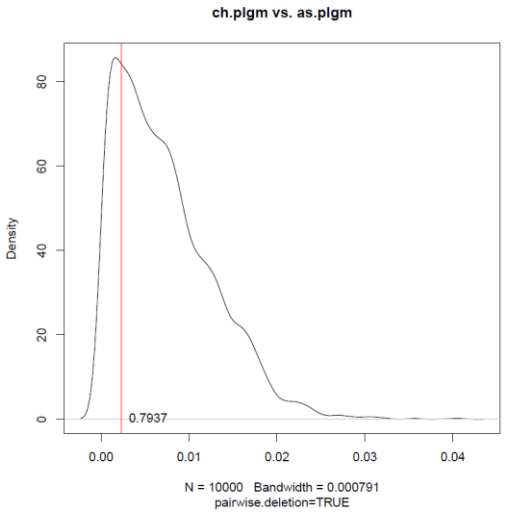

ch.plgm vs. ur.blgm
Density
0.9343
N = 10000 Bandwidth = 0.0003615
pairwise.deletion=TRUE

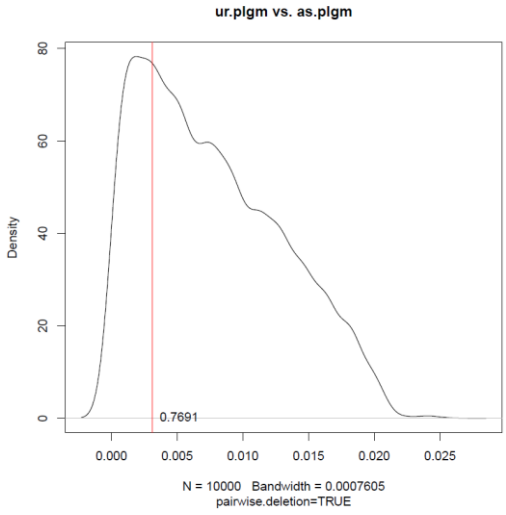

ch.plgm vs. ur.lgm
Density
0.864
N = 10000 Bandwidth = 0.0008102
pairwise.deletion=TRUE

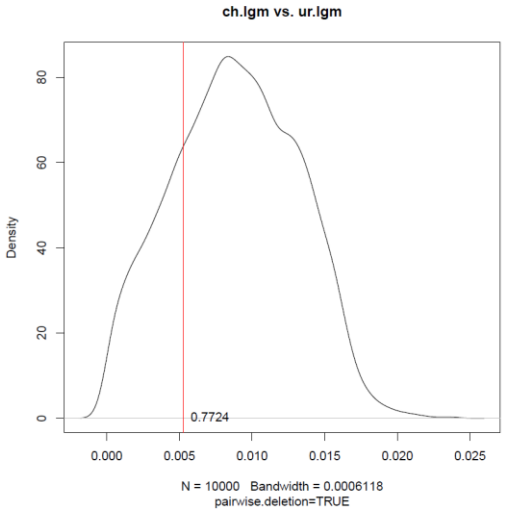

ch.plgm vs. as.lgm
Density
0.3771
N = 10000 Bandwidth = 0.0005798
pairwise.deletion=TRUE

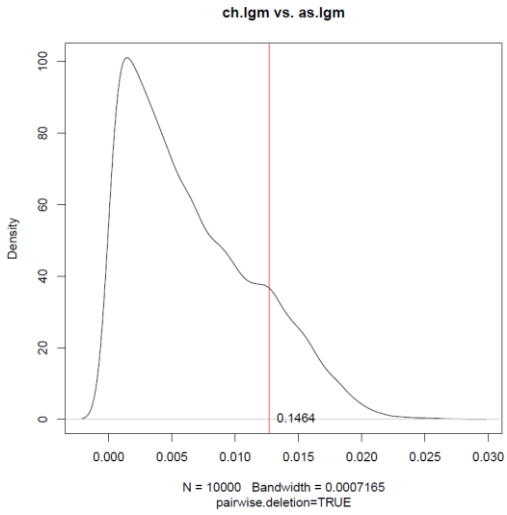

ch.plgm vs. as.blgm
Density
0.448
N = 10000 Bandwidth = 0.0003009
pairwise.deletion=TRUE

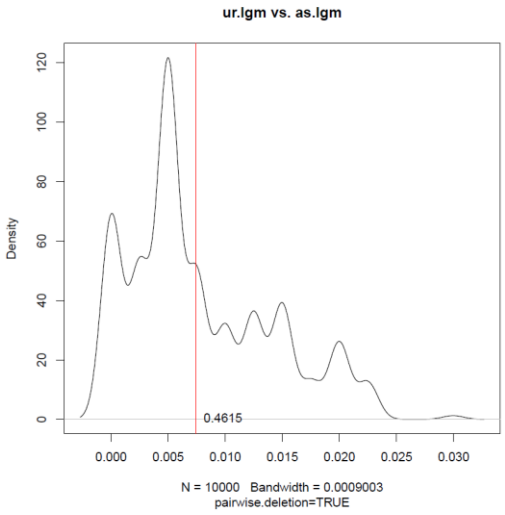

ch.plgm vs. ur.plgm
Density
0.8913
N = 10000 Bandwidth = 0.0004957
pairwise.deletion=TRUE

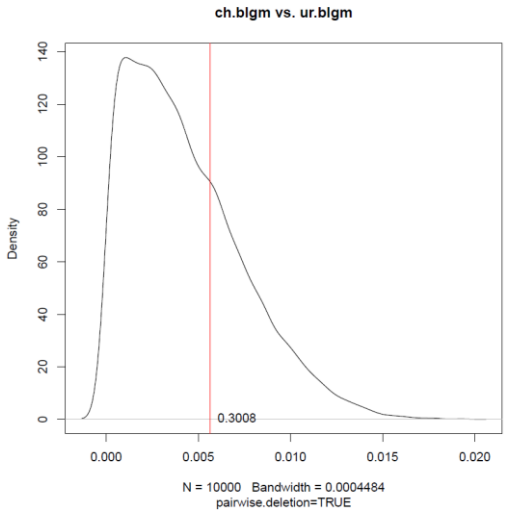

ch.plgm vs. as.plgm
Density
0.7937
N = 10000 Bandwidth = 0.000791
pairwise.deletion=TRUE

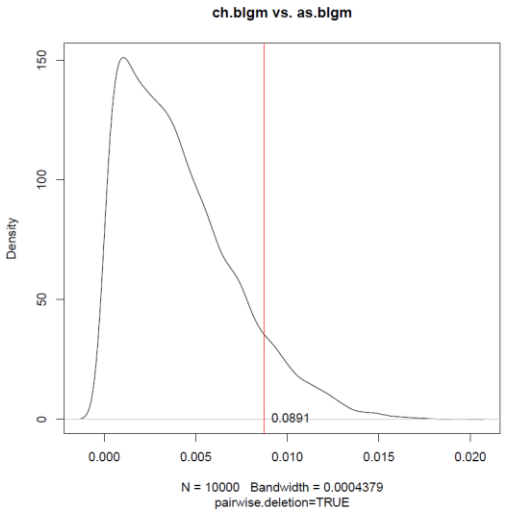

ur.plgm vs. as.plgm
Density
0.7691
N = 10000 Bandwidth = 0.0007605
pairwise.deletion=TRUE

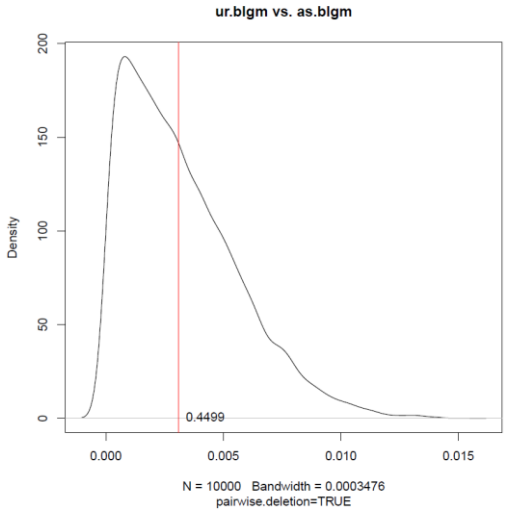

ch.lgm vs. ur.lgm
Density
0.7724
N = 10000 Bandwidth = 0.0006118
pairwise.deletion=TRUE


ch.lgm vs. as.lgm
Density
0.1464
N = 10000 Bandwidth = 0.0007165
pairwise.deletion=TRUE

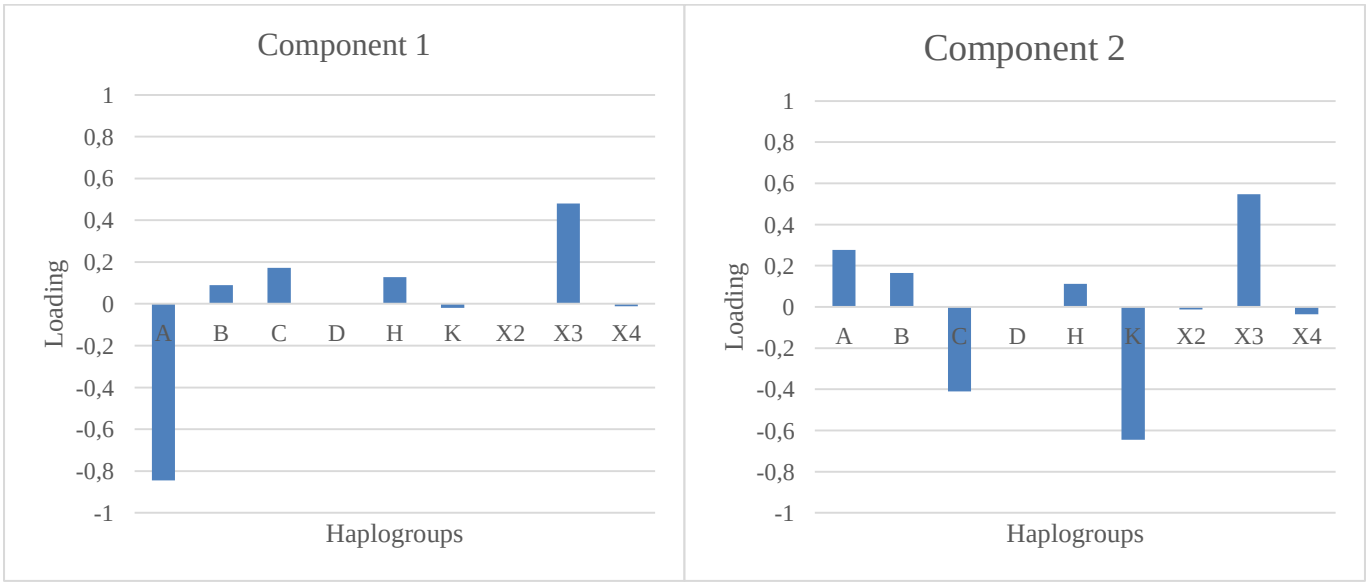

ur.lgm vs. as.lgm
Density
0.4615
N = 10000 Bandwidth = 0.0009003
pairwise.deletion=TRUE


ch.blgm vs. ur.blgm
Density
0.3008
0.000
0.005
0.010
0.015
0.020
N = 10000 Bandwidth = 0.0004484
pairwise.deletion=TRUE


ch.blgm vs. as.blgm
Density
0.0891
0.000
0.005
0.010
0.015
0.020
N = 10000 Bandwidth = 0.0004379
pairwise.deletion=TRUE


ur.blgm vs. as.blgm
Density
0.4499
0.000
0.005
0.010
0.015
N = 10000 Bandwidth = 0.0003476
pairwise.deletion=TRUE

S4 Fig: Influential haplogroups (loadings) of component 1 (left panel) and 2 (right panel) for PCA graph.



S1 Table: Details of investigated sites, including site context, main references, location, laboratory and archaeological code, skeletal element, GenBank accession code, and dates [extended from 29].

	Name of site	Site context	Reference	Location (alt., lat., lon.)	Samples					Age			
					Lab code	Arch code	Skeletal element	Genbank accession code	Haplogroup	Direct from sample		From other specimens	
										BP	calBP	BP	calBP
1	Schalbergfelsenhöhle	Limestone cave used by different species and at different times.	(57)	425m, 47.47, 7.57	PS1	Sch.795	Pre(Molar)	KC893841	A	ETH39763 34980±330	40032±856		
					PS2	Sch.833	Pre(Molar)	KC893842	A	ETH49373 32935±268	37414±676		
					PS4	Sch.806	Pre(Molar)	KC893843	X3	ETH39764 35995±320	41344±296		
					PS5	Sch.805	Pre(Molar)	KC893844	B	ETH49374 10544±41	12511±140		
2	Kohlerhöhle	Cave dwelling with two distinct cultural layers covered with sinter.	(58, 59)	378m, 47.43, 7.57	KOH1 (KOHI*)	A.3.L.	Premolar 3/4 inf. sin.	KC893804	B	ETH39760 11525±60	13423±117	B-4971 11640±150	13533±188[b]
					KOH2 (KOHI*)	A.3.L.	Molar 1/2 inf. dext.					B-4969 12820±160	15401±471[b]
					KOH3 (KOHI*)	A.3.L.	Molar 1/2 inf. sin.					ETH-43309 12460±45	14751±297[c]
					KOH5 (KOHI*)	A.3.L.	Molar 3 inf. dext.					ETH-43310 16205±55	19350±289[c]
					KOH6 (KOHII*)	-	Incisive dec.	KC893805	A	ETH39761 12790±45	15270±248		
					KOH9 (KOHII*)	H.a.	Premolar dec.3 inf. dext.						
					KOH13 (KOHIII*)	-	Incisive	KC893806	B	ETH39762 12465±40	14761±293		
					KOH18 (KOHIII*)	-	Premolar 2 sup. dext.						
					KOH4	A.6.r	Molar 3 inf. sin.	KC893807	D				
					KOH7	H.a.g.	Incisive	KC893808	A				
					KOH8	H.a.	Incisive	KC893809	A				
					KOH10	H.a.	Incisive	KC893810	K				
					KOH11	P.2.L.	Incisive	KC893811	A				

					KOH12	H.a.	Incisive	KC893812	A				
					KOH17	-	Premolar 3/4 dec. sup. sin.	KC893813	B				
					KOH19	-	Maxilla dext., Premolars 2-3, Molars 1-3	KC893814	H				
					KOH20	Prof. 13-14	Maxilla sin., Premolars 2-3, Molars 1-3	KC893815	H				
					KOH21	Prof. 9-10 L.o.	Molar sup.	KC893816	X3				
					KOH22	Prof. 14-16 Sch. I	Molar sup.	KC893817	X3	ETH44383 19615±75	23406±338		
					KOH23	P.12.L.u.	Molar sup.	KC893818	X3	ETH44381 19810±65	23721±275		
					KOH24	P.11.L.u.	Molar sup.	KC893819	X3	ETH44380 19970±70	23897±315		
					KOH25	Prof. 14-16 Sch. II	Molar sup.	KC893820	X3				
					KOH26	P.1.L.u.B.	Molar sup.	KC893821	A	ETH44378 12765±40	15223±258		
					KOH27	Prof. 13 M-u.	Molar sup.	KC893822	X3	ETH44382 19730±75	23610±251		
					KOH28	Prof. 14-16 Sch. II	Molar sup.	KC893823	X3				
					KOH29	P.12 L.u.	Molar sup.	KC893824	B				
					KOH30	Prof. 10 R.m.	Molar sup.	KC893825	X3	ETH44379 19305±75	23067±265		
					KOH31	P.0-1	Molar sup.	KC893826	A				
3	Kesslerloch	Limestone cave dwelling, layers covered with sinter.	(60-63)	440m, 47.75, 8.69	KES1	KESLH 33	Metapodial	KC893761	X4b			B-3327 11220±180	13120±198[d]
					KES2	KESLH 33.2	Metapodial	KC893762	X4b			KIA-33350 12225±45	14277±239[e]
					KES3	KESLH 26 II s	Incisive	KC893763	B			KIA-33351 12335±45	14502±338[e]
					KES5	KESLH 21 II c	Incisive	KC893764	X4b			KIA-11826 12502±52	14819±295[e]

	KES8	KESLH 33_21	Metapodial	KC893765	A/B/C/D/F			OxA-5748 12770±90	15213±302[f]
	KES9	KESLH 33_22	Metapodial	KC893766	B			KIA-11825 12774±54	15237±262[e]
	KES10	KESLH 38_44	Metapodial	KC893767	A/B/C/D/F			KIA-11829 12897±53	15591±329[e]
	KES11	KESLH 38_45	Metapodial	KC893768	X4b			Hv-10652 12890±90	15595±380[g]
	KES13	KESLH 38	Metapodial	KC893769	K			B-3329 12970±180	15798±512[d]
	KES16	KESLH 38	Metapodial	KC893770	A/B/C/D/F			KIA-11827 13052±53	15941±390[e]
	KES17	KESLH 11	Incisive	KC893771	X4b			OxA-5746 13120±90	16038±401[f]
	KES18	KESLH 26	(Pre)molar M3/P2	KC893772	X3			OxA-5747 13430±100	16367±427[f]
	KES19	KESLH 26	(Pre)molar M3/P2	KC893773	X4b			OxA-5750 13670±100	16745±270[f]
	KES20	KESLH 26	(Pre)molar M3/P2	KC893774	B			KIA-11828 13858±55	17085±180[e]
	KES21	KESLH 26	(Pre)molar M3/P2	KC893775	H			OxA-10239 13980±110	17234±228
	KES27	KESLH 38	Molar 3 inf. dext.	KC893776	B			OxA-5749 14150±100	17392±254[f]
	KES29	KESLH 36 3 B s	Molar 3 inf. dext.	KC893777	A	ETH44387 13035±60	15915±396	OxA-10238 14330±110	17505±261[e]
	KES31	KESLH 32 III A c	Molar sup.	KC893778	A			OxA-10298 15020±180	18254±263[e]
	KES34	KESLH 13 I s	Molar sup. sin.	KC893779	A/B/C/D/F	ETH44388 12605±50	14962±295		
	KES35	KESLH 34 III B c lower part	Molar inf.	KC893780	A/B/C/D/F				
	KES37	KESLH 34 III B c	Molar sup.	KC893781	B				
	KES38	KESLH 34 III B c	Molar sup.	KC893782	A/B/C/D/F				
	KES39	KESLH 26 II s	Molar inf.dext.	KC893783	X4b				

	KES41	KESLH 11; HE11	Molar sup. dext.	KC893784	B	ETH44385 13690±50	16824±201
	KES42	KESLH 34 III B c	Molar sup.	KC893785	D		
	KES43	KESLH 34_20	Metapodial	KC893786	A/B/C/D/F		
	KES46	KESLH 34; HE34	Molar inf., small	KC893787	A/B/C/D/F		
	KES47	KESLH 26 II s	Molar sup.	KC893788	X4b		
	KES48	KESLH 26 II s	Molar sup.	KC893789	A		
	KES49	KESLH 24 II u	Metapodial	KC893790	B		
	KES50	KESLH 24 II u	Metapodial	KC893791	K		
	KES51	KESLH 12 I u	Metapodial	KC893792	H		
	KES53	KESLH 33 III A s	Molar sup. dext.	KC893793	K	ETH44384 12885±65	15560±333
	KES54	KESLH 24; HE 24 II u	Molar inf. sin.	KC893794	X4b		
	KES55	KESLH 24; HE 24 II u	Molar inf. sin.	KC893795	X4b		
	KES56	KESLH 24; HE 24 II u	Molar inf. sin.	KC893796	A/B/C/D/F		
	KES58	KESLH 33 III A s	Molar sup. sin., small	KC893797	X3		
	KES59	KESLH 34 III B c	Molar inf. dext.	KC893798	B		
	KES61	KESLH AG22	Metacapal small	KC893799	A/B/C/D/F		
	KES62	KESLH AG19	Metatarsal	KC893800	K/X4b		
	KES63	KESLH 21 II c	Molar inf.	KC893801	A		

					KES66	KESLH 24 II n	Molar sup. sin.	KC893802	H	ETH44386 12795±55	15277±254		
					KES67	KESLH 24 II n	Molar sup. sin.	KC893803	X3				
4	Käsloch	Limestone cave dwelling.	(64, 65)	420m, 47.37, 7.91	KL1	127 6 7138	Molar	KC893827	B	ETH39769 12505±45	14825±291		
					KL3	127 6 7102	Molar	KC893828	A/B/C/D/F	ETH39770 13760±45	16931±154		
					KL4	127 6 7140	Molar	KC893829	H	ETH39771 12450±45	14719±313		
5	Rislisberghöhle	Limestone cave dwelling.	(66, 67)	488m, 47.3, 7.7	RIS3	707	Dec. molar inf. sin.	KC893831	K	ETH39768 10770±45	12749±50	Ly-1099 11860±230	13868±346[b]
					RIS6	103/28 Fk252 Feld66	(Pre)molar	KC893832	K			ETH-42516 12680±45	15064±301[c]
					RIS7	103/28 Fk1133	(Pre)molar	KC893833	K			ETH-42515 12710±45	15112±296[c]
					RIS13	103/28 Fk1130	(Pre)molar	KC893834	K	ETH44377 12575±55	14924±293	ETH-42517 13000±50	15856±398[c]
6	Schweizersbild	Rockshelter.	(68, 69)	472m, 47.72, 8.64	SB1	Yellow cultural layer	Molar inf.	KC893835	B				
					SB2	Yellow cultural layer	Molar inf.	KC893836	B				
					SB6	Yellow cultural layer	(Pre)molar inf.	KC893837	A	ETH44390 12240±60	14308±258		
					SB7	-	Molar inf.	KC893838	B	ETH44392 12690±50	15077±33		
					SB10	-	Molar sup.	KC893839	A				
					SB11	Lower rodent layer	Molar sup.	KC893840	X3	ETH44391 12240±50	14303±251		
7	Abri Neumühle	Rockshelter.	(70)	520m, 47.44, 7.33	RAN1	NM o.B. 89.1.A	Molar	KC893830	C	ETH44393 12305±40	14439±324		
8	Twann-Bahnhof	Lacustrine settlement,	(71)	426m, 47.09,7.17	TWB3	1009.6	Metatarsus III dext. dist.	KC893755	B	ETH39773 4800±35	5540±46	dendrochronological layer date 3666±35 BC	

		transgressed.			TWB4	167	Phalanx I post. dext., Phalanx II, Humerus prox. Epiphysis sin.	KC893756	D			dendrochronological layer date 3598±25 BC
					TWB5	909.21 Layer E6	Phalanx I ant. sin.	KC893757	B	ETH39774 4740±35	5467±97	dendrochronological layer date 3598±25 BC
					TWB9	1723.5	Premolar dec.2 inf. dext.	KC893758	B			dendrochronological layer date 3666±35 BC
					TWB10	1247 V-10	Scapula sin. dist.	KC893759	A/B/C/D/F			dendrochronological layer date 3666±35 BC
					TWB13	1127 V-10	Sesamoid	KC893760	A			dendrochronological layer date 3666±35 BC
9	Mumpf	Settlement.	(72)	287m, 47.55, 7.92	MU1	123	Premolar 3/4 sup.	KC893753	D	ETH36444 4525±55	5180±101[i]	
					MU2	-	Molar 3 sup.	KC893754	D	ETH36446 4395±60	5042±139[i]	

[a]Bodenforschung Basel-Stadt, [b][73], [c][74], [d][75], [e][63], [f][76], [g][77], [i][72].
*Multiple teeth presumably belonging to the same individual.

S2 Table: Parameters for weighting of nucleotide positions for Median Joining Network analysis based on 97 Pleistocene horse mitochondrial d-loop sequences.

	1	3	4	5	20	28	30	35	42	43	45	49	51	53	59	60	67	74	82	90	92	93	94	96	104	106	107	109	111	112	
Positions in reference sequence																															
NC_001640	15492	15494	15495	15496	15511	15519	15521	15526	15533	15534	15536	15540	15542	15544	15550	15551	15558	15565	15573	15581	15583	15584	15585	15587	15595	15597	15598	15600	15602	15603	
Nucleotide sequence of majority	A	T	C	A	G	C	G	T	A	C	T	A	C	T	C	C	G	C	T	G	A	C	A	C	A	A	T	G	T	T	
Number of changes to A					1		1										1			1								1			
Number of changes to T			1			1				1				14	3	1		1				1		1							
Number of changes to C		1						2			7				6				1								1		4	1	
Number of changes to G				1					1			8									1		56		1	8					
Number of missing nucleotides	5															4	4	10	6	6	6	6	7	6							
Number of transitions		1	1	1	1	1	1	2	1	1	7	8	14	6	3	1	1	1	1	1	1	1	56	1	1	8	1	1	4	1	
Number of transversions																															
In European dataset (77)			1		1	1				1	7	6	13	4	2	1			1	1	1	1	42	1		4			3		
In Ural dataset (13)							1	1				2	1	1	1			1					8			1		1			
In Asian dataset (15)		1		1				1	1					1			1						6		1	3	1		1	1	
Total		1	1	1	1	1	1	2	1	1	7	8	14	6	3	1	1	1	1	1	1	1	56	1	1	8	1	1	4	1	
Calculated weight	0	49	49	49	49	49	49	48	49	49	43	42	36	44	47	49	49	49	49	49	49	49	0	49	49	42	49	49	46	49	
Actual weight in MJN	**0**	**49**	**49**	**49**	**49**	**49**	**49**	**48**	**49**	**49**	**43**	**42**	**50**	**44**	**47**	**49**	**49**	**49**	**49**	**49**	**49**	**49**	**0**	**49**	**49**	**0**	**49**	**49**	**46**	**49**	
	113	119	120	121	126	132	144	151	155	158	159	160	163	168	175	176	212	218	221	227	229	235	249	256	262	263	264	265	266	267	
Positions in reference sequence																															
NC_001640	15604	15610	15611	15612	15617	15623	15635	15642	15646	15649	15650	15651	15654	15659	15666	15667	15703	15709	15712	15718	15720	15726	15740	15747	15753	15754	15755	15756	15757	15758	
Nucleotide sequence of majority	G	T	G	A	T	T	C	C	C	A	A	G	C	T	G	A	T	C	A	C	A	G	A	A	G	G	G	A	A	A	
Number of changes to A	16		1					1				1			10							1			1						37
Number of changes to T							4	1	1				1					1		5											38
Number of changes to C		1			21	1								12			17														75
Number of changes to G				1						5	41					1			1		2		3	4							134
Number of missing nucleotides															14	14										1	1	6	6	6	
Number of transitions	16	1	1	1	21	1	4	1	1	5	41	1	1	12	10	1	17	1	1	5	2	1	3	4	1						283
Number of transversions								1																							1
In European dataset (77)	10	1	1		19	1	4	2	1	2	30		1	11	8		8	1		2	2		3		1						
In Ural dataset (13)	2			1	1					1	4				1		4			2											
In Asian dataset (15)	4				1					2	7	1		1	1	1	5			1		1		4							
Total	16	1	1	1	21	1	4	2	1	5	41	1	1	12	10	1	17	1	1	5	2	1	3	4	1						284
Calculated weight	34	49	49	49	19	49	46	48	49	45	7	49	49	38	40	49	33	49	49	45	48	49	47	46	49	0	0	0	0	0	
Actual weight in MJN	**0**	**49**	**49**	**49**	**50**	**49**	**46**	**48**	**49**	**45**	**0**	**49**	**49**	**50**	**50**	**49**	**0**	**49**	**49**	**45**	**48**	**49**	**47**	**46**	**49**	**0**	**0**	**0**	**0**	**0**	

S3 Table: Details of haplogroups detected in Pleistocene horses from the Swiss and Swabian Jura region, nomenclature follows [2]. Haplogroup defining nucleotide positions relative to the horse reference mitogenome [1] are shown according to their position. All deviations from the reference sequence are given, with mandatory defining positions in bold and optional nucleotide positions in parenthesis. Note that transitions on nucleotide positions 15,585; 15,604 and 15,650 occur sporadically in all haplogroups; these positions are regarded as hotspots and therefore dismissed.

Haplogroup	Nucleotide positions
A	**15,495; 15,602; 15,720**
B	15,495; 15,602; **15,617**; (**15,635**); (**15,649**); (**15,659**); 15,720
C	15,495; 15,602; (**15,649**); 15,720
D	15,495
H	15,495; **15,536**; 15,602; 15,720
K	15,495; 15,602; **15,703**; 15,720; (**15,740**)
X3	15,495; **15,542**; (**15,544**); 15,602; **15,666**; 15,720
X4b	15,495; **15,540**; 15,602; (**15,718**); 15,720

S4 Table: Nucleotide and haplotype diversities in horse populations from Switzerland and the Swabian Jura (all datasets).

Time period	Dataset 1				Dataset 2				Dataset 3			
	Number of samples	Number of haplotypes	Nucleotide diversity	Haplotype diversity	Number of samples	Number of haplotypes	Nucleotide diversity	Haplotype diversity	Number of samples	Number of haplotypes	Nucleotide diversity	Haplotype diversity
Palaeontological	4	3	0.0111	0.83	4	4	0.0104	1	4	4	0.0104	1
Badegoulian	11	3	0.0089	0.47	11	4	0.0093	0.6	5	1	0	0
Magdalenian	70	14	0.0092	0.63	53	28	0.016	0.95	20	8	0.0091	0.7
Magd. + Azilian	74	14	0.0103	0.63	57	29	0.0159	0.95	-		-	-
Azilian	4	2	0.0111	0.67	4	3	0.0104	0.83	3	2	0.0111	0.67
Neolithic	8	3	0.0086	0.68	6	2	0.0149	0.6	4	2	0.0166	0.67

S5 Table: F_{ST} values of pairwise populations (all datasets). Lower triangle: F_{ST} values, upper triangle: *p* values. Comparable populations are boxed, significant F_{ST} values are in bold.

	Dataset 1						Dataset 2						Dataset 3				
	Palaeontological	Badegoulian	Magdalenian	Magd.+ Azilian	Azilian	Neolithic	Palaeontological	Badegoulian	Magdalenian	Magd.+ Azilian	Azilian	Neolithic	Palaeontological	Badegoulian	Magdalenian	Azilian	Neolithic
Palaeonto-logical	-	.009	.3	.3	.4	.1	-	.01	.6	.5	.2	.1	-	.008	.5	.08	.2
Badegoulian	**.4**	-	0	.0002	.003	.001	**.3**	-	.0003	.0003	.002	.0003	**.77**	-	0	.01	.008
Magda-lenian	.01	**.28**	-	.8	.05	.05	0	**.16**	-	1	.1	.0008	0	**.68**	-	.03	.003
Magd.+ Azilian	.009	**.26**	0	-	.1	.07	0	**.17**	0	-	.2	.0006	-	-	-	-	-
Azilian	.22	**.5**	.17	.09	-	.06	.17	**.46**	.06	.04	-	.1	.21	**.85**	.25	-	.2
Neolithic	.13	**.45**	.07	.07	.33	-	.21	**.5**	**.2**	**.2**	.26	-	.17	**.78**	**.29**	.21	-

S6 Table: Tajima's *D*, Fu's F_S, sum of squared deviances (SSD) and Harpending's raggedness index results for horse populations from Switzerland and the Swabian Jura (all datasets). Significant results are in bold. NaN = not a number because only one haplotype was present.

	Dataset 1								Dataset 2								Dataset 3							
	Tajima's *D*	*p*	Fu's F_S	*p*	SSD	*p*	Raggedness index	*p*	Tajima's *D*	*p*	Fu's F_S	*p*	SSD	*p*	Raggedness index	*p*	Tajima's *D*	*p*	Fu's F_S	*p*	SSD	*p*	Raggedness index	*p*
Palaeontol.	-0.21	.6	0.56	.5	**0.27**	0.03	0.75	0.2	-0.8	.2	-1.51	.06	0.03	0.8	0.11	0.9	-0.8	.2	**-1.51**	.05	0.03	0.8	0.11	0.9
Badegoulian	0.19	.6	2.34	.9	**0.33**	0.0007	0.4	0.9	-0.09	.5	0.77	.7	0.13	0.1	0.33	.02	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN
Magdalenian	**-1.53**	.04	**-8.39**	.0004	**0.24**	0.0003	0.03	1	-1.32	.07	**-21.71**	0	0.001	0.6	0.02	0.6	**-1.53**	.049	-2.3	.07	0.07	0.1	0.25	0.09
Magd.+Azil.	-1.35	.07	**-7.29**	.001	0.02	0.4	0.1	0.5	-1.29	.08	**-22.59**	0	0.001	0.63	0.02	0.6	-	-	-	-	-	-	-	-
Azilian	2.08	1	2.72	.9	**0.37**	0.02	1	0.3	1.37	.9	0.46	.5	0.08	0.33	0.25	0.7	0	.8	2.02	.8	0.37	0.05	1	0.6
Neolithic	0.58	.8	0.72	.6	0.06	0.2	0.23	0.3	2.12	1	4.51	1	**0.38**	0.02	**0.88**	0.03	2.16	1	3.53	.9	**0.41**	0.02	1	0.3

S7 Table: Sequences of published Pleistocene horses from Eurasia. Sequences marked with an '[a]' are part of draft full genomes which are obtainable as SRA-Illumina runs on GenBank.

Origin	Date	GenBank accession code	Reference
Germany (south)	50-14,000 BP	DQ007558/DQ007611; DQ007556/DQ007609; DQ007591; DQ007590	Weinstock et al. (2005)
		FJ204352	Cieslak et al. (2010)
Russian Federation (Taymyr peninsula)	43-16,000 BP	upon request [a]	Orlando et al. (2013)
Russian Federation (Novosibirsk islands, Sakha Republic)	40-20,000 BP	upon request [a]	Orlando et al. (2013)
		JN570964-JN570966	Lorenzen et al. (2011)
		FJ204314-FJ204318	Cieslak et al. (2010)
Russian Federation (Lena river delta, Sakha Republic)	36-31,000 BP	JN570958; JN570959	Lorenzen et al. (2011)
		DQ007577	Weinstock et al. (2005)
Russian Federation (Kolyma lowlands, Sakha Republic)	38-17,000 BP	JN570957; JN570961	Lorenzen et al. (2011)
Russian Federation (Yana river basin, Sakha Republic)	22,500 BP	JN570960	Lorenzen et al. (2011)
Russian Federation (Urals)	46-17,000 BP	JN570954; JN570972-JN570982; JN570993	Lorenzen et al. (2011)

S8 Table: Nucleotide and haplotype diversity of Eurasian Pleistocene horses based on pairwise deletion of missing nucleotides.

Region	Time bin	Number of sequences	Number of haplotypes	Nucleotide diversity	Haplotype diversity
Asia	BLGM	11	6	0.0164	0.8
	LGM	3	2	0.015	0.67
	PLGM	2	2	0.0187	1
Urals	BLGM	7	5	0.0153	0.9
	LGM	2	2	0.015	1
	PLGM	4	3	0.0137	0.83
Switzerland	BLGM	4	4	0.0104	1
	LGM	11	4	0.0093	0.6
	PLGM	57	29	0.0159	0.95

S9 Table: F_{ST} values of Eurasian Pleistocene horses. Lower triangle: F_{ST} values, upper triangle: *p* values. Comparable populations are boxed, significant F_{ST} values are in bold. Swiss/Swabian samples: dataset 2.

A	Asia BLGM	Asia LGM	Asia PLGM	Ural BLGM	Ural LGM	Ural PLGM	Swiss BLGM	Swiss LGM	Swiss PLGM
Asia BLGM	-	0.3	0.6	0.5	0.6	0.5	0.7	**0.0008**	**0.03**
Asia LGM	0	-	0.4	0.4	0.8	0.3	0.4	**0.03**	0.09
Asia PLGM	0.068	0.055	-	0.3	1	0.3	0.07	**0.05**	0.1
Ural BLGM	0	0.01	0.103	-	0.4	0.6	0.7	**0.005**	0.3
Ural LGM	0	0	0	0	-	0.1	0.2	**0.01**	0.1
Ural PLGM	0	0.076	0.144	0.032	0.255	-	1	0.2	0.8
Swiss BLGM	0	0.031	0.203	0	0.203	0	-	**0.02**	0.5
Swiss LGM	**0.233**	**0.383**	**0.387**	**0.265**	**0.51**	0.162	**0.297**	-	**0.0003**
Swiss PLGM	**0.05**	0.11	0.142	0.01	0.163	0	0	**0.167**	-

2.3 Elsner J, Deschler-Erb S, Stopp B, Schibler J, Hofreiter M, Schlumbaum A (2016) Mitochondrial d-loop variation, coat colour and sex identification of Late Iron Age horses in Switzerland. *J Archaeol Sci: Rep* 6:386-396. doi:10.1016/j.jasrep.2016.03.007

Journal of Archaeological Science: Reports 6 (2016) 386–396

Contents lists available at ScienceDirect

Journal of Archaeological Science: Reports

journal homepage: www.elsevier.com/locate/jasrep

Mitochondrial d-loop variation, coat colour and sex identification of Late Iron Age horses in Switzerland

Julia Elsner [a,*], Sabine Deschler-Erb [a], Barbara Stopp [a], Michael Hofreiter [b], Jörg Schibler [a], Angela Schlumbaum [a]

[a] Integrative Prehistory and Archaeological Science, Department of Environmental Sciences, Basel University, Spalenring 145, 4055 Basel, Switzerland
[b] Institute for Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Str. 24–25, D-14476 Potsdam, Germany

ARTICLE INFO

Article history:
Received 26 November 2015
Accepted 7 March 2016
Available online 14 March 2016

Keywords:
Genetic diversity
Ancient DNA
Iron Age
Horse
Coat colour
Withers height
Greying
Sex identification

ABSTRACT

In the Celtic world, horses enjoyed a prominent position as status symbols and objects of veneration, yet little is known about these Celtic horses except that they were rather small. The Late Iron Age was a time defined by increasing inter-cultural contact between Celtic peoples and the Romans. This is, amongst other features, observable in the phenotypes of domestic livestock such as horses. Amongst the usually small animals, larger ones are rarely but regularly encountered in the archaeological record. We have investigated mitochondrial (mt) DNA d-loop diversity, sex and coat colour using bones from 34 horses of different size from three Swiss sites (Mormont, Basel-Gasfabrik, Aventicum) most of them dating from 150 to 50 BCE. The aim was to characterise the diversity of matrilineages and coat colourations of Iron Age horses, and to identify molecular sex. We detected eleven mt haplotypes clustering into six haplogroups (B, D, F, I, X2, X3) in the ancient dataset (n = 19). Large individuals were all male, but smaller stallions were also identified; molecular sexing confirmed and augmented to morphological results. The horses were bay, chestnut and black in colour, and spottings or dilutions were absent in all animals. With a simplified primer system to detect premature greying, white coats can be excluded as well. The limited colour range proposes selection for monochrome animals. Additionally, ancient matrilineages were compared to modern horses from regions appertaining to the Late Roman Republic and to European pony breeds. Based on Principal Component Analysis (haplotype frequencies) and F_{ST}-values (genetic distances) the mtDNA variation of the Iron Age horses investigated here has survived in modern European breeds, particularly in northern European ponies.

1. Introduction

Several Late Iron Age sites have been identified in Switzerland, including the eponymous sacrificial site in La Tène, canton Neuchâtel. In recent years comprehensive interdisciplinary investigations were carried out at Basel-Gasfabrik (Pichler et al., in preparation) and on the hill of Mormont (Brunetti et al., 2014) to gain detailed insight into the socio-economy of Celtic people in the region. In the Late Iron Age, contacts between Celtic groups and Romans intensified. Mainly the Celtic nobility were susceptible for Roman influence and long distance trade increased in the alpine foreland (Brem and Haldimann, 1999) to import prestige items like wine and tableware, and, according to Caesar, also horses (bell. gall. 4,2,2: Möller, 2013). Several written sources describe ancient horse and mule trade across Europe, Asia and Africa (see e.g. Peters, 1998: 140f), yet the scientific evidence for this is scarce (Paulus and Uerpmann, 2007; Nuviala et al., 2014). Horses were venerated status symbols in ancient times (Green, 1992); they were significant for transportation and warfare; however, horse meat was also eaten on a regular basis by the Celts. Horses played an important part in Celtic symbolism as well, apparent in Epona, patroness of horses and riders, and in the ritual life as indicated for example from perforated horse skulls from La Tène (Méniel, 2007) or carcass depositions in Gournay-sur-Aronde, France (Rapin et al., 1980). However, most Iron Age sites in Switzerland do not contain more than 5% horse remains (Schibler et al., 1999: 121). Celtic horses are described as small (110–130 cm withers height), whereas the Romans also bred larger animals (>140 cm) (Müller-Lhotska, 1984: 114; Peters, 1998: 149f; Arbogast et al., 2002: 44f). Therefore, when encountering small and large horses in Celtic contexts, archaeozoologists often consider the small ones to be local, while larger ones are regarded as imported, endowed or looted. Some authors argue that the reason for the small size of the Celtic horse was poor pasture and low breeding interest (Boessneck et al., 1971: 106), but this interpretation contradicts the Celts' reputation as stock breeders and mounted warriors (Hyland, 1990: 173; Green, 1992: 66; Sidnell, 2006: 162). As small horses are more versatile and quick on the battlefield (Junkelmann, 1990: 44), it can be assumed that the Celts intentionally bred small horses. However, by the Late Iron Age, small and large horses were found to be coexisting in Celtic sites for example in southern Germany (Manching) or Luxembourg (Titelberg)

* Corresponding author.
E-mail address: julia.elsner@email.de (J. Elsner).

http://dx.doi.org/10.1016/j.jasrep.2016.03.007

(Boessneck et al., 1971; Méniel, 2014: 18). Little is known about the genetic diversity of Celtic and Roman horses (Di Bernardo et al., 2004; Bower et al., 2013), yet it is speculated that the Romans introduced new female lineages to conquered regions (Bower et al., 2013).

In ancient DNA (aDNA) studies, the analysis of the non-recombining maternally inherited mitochondrial (mt) DNA has been widely applied due to its high copy number in the cell and the high variability within the non-coding displacement-loop (d-loop). Studies of modern horse mtDNA diversity have revealed an intricate phylogenetic structure. Based on 616 base pairs six major haplogroups were defined (Vila et al., 2001; Jansen et al., 2002). The inclusion of additional aDNA data (Cieslak et al., 2010) and whole mitochondrial genomes (Lippold et al., 2011b; Achilli et al., 2012) led to revisions of the initial nomenclature, resulting in now 18 or 19 haplogroups. Apparently, geographic structuring is low today, and was so in wild horse populations, but there is also evidence that some matrilineages were regionally confined (Cieslak et al., 2010; Lira et al., 2010). Studies featuring ancient horse mtDNA suggest multiple domestications and an increasing diversity of female lineages from the Bronze Age onwards (Vila et al., 2001; Cieslak et al., 2010).

Animals with unique colour variants may have been specially valued. Individual phenotypic information can be retrieved from nuclear DNA, e.g. from single nucleotide polymorphisms (SNPs). An increasing number of genetic loci associated with coat colour has been detected so far (for reviews see Rieder, 2009; Cieslak et al., 2011) including the pleiotropic effect some of them have on behaviour and health (Reissmann, 2009: 18f; Trut et al., 2009; Bellone, 2010).

Morphological sex identification in archaeological horse remains is feasible when determinant skeletal elements are preserved; these include the canines, which dominantly prevail in males, and the pelvis (Montane et al., 1913). Molecular sex identification is possible using the amelogenin gene (Iwase et al., 2003) and the zinc-finger gene on the X and Y chromosomes (Senese et al., 1999; Han et al., 2010).

Here we investigate horse remains, mostly with known withers heights, from three key Late Iron Age sites in Switzerland where Roman cultural influence is noticeable in the archaeological record. We explore mitochondrial d-loop variation, molecular sex and coat colour markers of 32 Late La Tène and two Roman time horses. The diversity of the ancient female lineages is compared to modern indigenous horse and pony breeds. This is the first study focusing on variation in horses from a defined region inhabited by Celtic peoples, zooming in on a narrow time frame.

2. Material and methods

2.1. Sites and material

We have selected 32 horse remains from Late Iron Age contexts (La Tène D, 150 BCE–30 CE), the sites of Mormont, Basel-Gasfabrik and pre-Roman Aventicum in Switzerland (Fig. 1). Two samples dating to the late 1st to early 2nd century AD from Aventicum have been added to the analysis. Sample details are given in Table 1. All sites comprised more horse remains than investigated here.

2.1.1. Mormont

The site on the hill of Mormont near Eclépens and La Sarraz, canton Vaud, was first discovered in 2006 during gravel works and addressed as a Helvetic sanctuary (Dietrich et al., 2007). After examining the faunal remains, a second scenario was proposed: the site might have been a temporal camp of refugees or migrants (Méniel, 2014: 195f). Since the investigations by other archaeological disciplines are not finished yet, both interpretations have to be regarded as preliminary. According to typological dating the main occupation time of the site was La Tène D1b (130/120–90/80 BCE), which is supported by absolute dendrochronological dates between 107 and 102 BCE (Kaenel, 2014). During this time c. 300 structures were constructed including 260 pits, filled with various items ranging from mill stones, ceramic and metal pots, and decorative objects to an abundance of faunal remains and even several human bones. Horses (n = 40) are the third most common animal based on minimum number of individuals (MNI), after cattle (n = 174) and pigs (n = 59) (Méniel, 2014: 12). Given the short occupation time, they might stem from contemporaneous populations. The dead animals had been treated in different ways, some were deposited as articulated (partial) skeletons or cadavers, other bones show cut marks

Fig. 1. Location of investigated sites in Switzerland. 1 – Mormont, canton Vaud; 2 – Avenches/Aventicum, canton Vaud; 3 – Basel-Gasfabrik, canton Basel-City.

Table 1
Details of archaeologic sites and samples, and summary of results. Given are name, location and date of sites, and laboratory and archaeological code, morphological sex and age identification if determinant elements (pelvis, teeth) are present, skeletal element for withers height estimation and genetic analyses, as well as mitochondrial haplotype, GenBank accession code of d-loop sequences; molecular sex and coat colour. The measurement for greatest length (GL) and smallest breadth of diaphysis (SD) are given in mm, withers height (WH) was estimated after May (1985) and is given in cm. For some of the samples from Mormont, radius GL and SD from the same individual are given in parenthesis (Méniel, 2014: 264). Details on the mitochondrial haplotypes are given in Table 5, on molecular sexing in Table 6 and nuclear loci for the coat colour analysis in Table 7. Nomenclature of mitochondrial haplotypes follows Cieslak et al. (2010).

Site, location (altitude, latitude, longitude), date	Samples										
	Lab code	Arch code	Morph. sex, age	Skeletal element	GL	SD	WH	Mitochondrial haplotype	GenBank ID	Molecular sex	Coat colour
Mormont, 605 m, 46.66/6.54, Late La Tène, c. 100 BCE	MO1	Id 4 fosse 293	Female, 18 y	Metacarpus sinister	191 (285)	27 (31)	116.5 (117)	I	KC893855	–	–
	MO2	Id 35 fosse 288	–	Metacarpus sinister	181	27	110.5	D3	KC893856	Female	–
	MO3	Id 3 fosse 42	Female, 11.5 y	Metacarpus sinister	191 (281)	25.5 (29.3)	116.5 (115.5)	–	–	–	–
	MO4	Id 30 fosse 143	–	Metacarpus sinister	199	29	121.5	D2	KC893857	–	Chestnut
	MO5	Id 21 fosse 94	Female, 15 y	Metacarpus sinister	209	30	127.5	X2b	KC893858	Female	Black/not grey
	MO6	Id 44 fosse 196	–	Metacarpus sinister	202	30.5	123	B1	KC893859	Male	Black
	MO7	Id 2 fosse 131	Female, 10 y	Metacarpus sinister	189 (281)	27 (30.5)	115 (115.5)	–	–	–	–
	MO8	Id 27 fosse 146	–	Metacarpus sinister	205	28	125	–	–	–	–
	MO9	Id 42 fosse 275	–	Metacarpus sinister	194	29	118	X3c1 + 15,615G	KC893860	Male	Black/not grey
	MO10	Id 01 fosse 45	Male, 10 y	Metacarpus dexter	241 (365)	36 (41)	147 (150)	A/B/C/D/F?	KC893861	–	–
	MO11	Id 10 fosse 146		Metacarpus sinister	Incomplete			–	–	–	–
	MO12	Id 32 fosse 146	–	Metacarpus sinister	190	28	116	F	KC893862	–	–
	MO13	Id 53 fosse 210	Male, 5.5 y	Metacarpus sinister	191 (290)	29.7 (32)	116.5 (119)	–	–	–	–
	MO14	Id 58 fosse 542	–	Metacarpus sinister	Incomplete			K?	KC893863	–	–
	MO15	Id 54 fosse 210	Male, 5 y	Metacarpus sinister	208	29.7	127	X2b	KC893864	–	–
	MO16	Id 16 fosse 205	Male, 4 y	Metacarpus sinister	234 (340)	35.5 (39.2)	143 (140)	F	KC893865	Male	Bay/not grey
	MO17	Id 57 fosse 542	–	Metacarpus sinister	194	30	118	X2b	KC893866	–	Chestnut/not grey
	MO18	Id 55 fosse 169	Male, 1.5 y	Metacarpus sinister	Incomplete (subadult)			X2	KC893867	–	–
Basel-Gasfabrik, 255 m, 47.57/7.58, Late La Tène, 150–80 BCE	BGF1	Fk22519 pit44 excavation 1990/42	–	Metacarpus	201.5	30.4	123	F	KC893850	–	Chestnut
	BGF2	Fk22576 pit284 excavation 1990/32	–	Metacarpus	222.8	35.5	136	X2b	KC893851	–	–
	BGF3	Fk"2" pit114 excavation 1941/4	–	Metacarpus	196.9	31.4	120	X3c1 + 631G	KC893852	–	–
	BGF4	Fk"2" pit114 excavation 1941/4	–	Metacarpus	178.1	24.9	109	–	–	–	Chestnut
	BGF5	Fk 18796 pit256 excavation 1989/5	–	Radius/Ulna	276	33	113.5	F	KC893854	–	–
	BGF6	Fk(18943)18714 pit255 excavation 1989/5	–	Upper (pre)molar		–		–	–	–	–
Avenches, 630 m, 46.87/7.05, *Late La Tène, c. 50 BCE, **Roman, late 1st to early 2nd century CE	AV1**	K10656, Sk4	–	Radius	330	35	135.5	–	–	–	–
	AV2*	K10574	–	Radius/ulna	315	36	129.5	X3c1 + 15,615G	KC893849	–	–
	AV3**	K10656, Sk3	–	Radius	358	39.7	147	X2	KC893848	–	–

* refers to AV2 and Late La Tène. ** refers to AV1, AV3 and Roman.

and traces of burning. We have examined 18 randomly chosen equid metacarpi in this study.

2.1.2. Basel-Gasfabrik

In Basel, a settlement of the Rauricii was discovered in 1911 at the left border of the river Rhine (Hecht and Niederhäuser, 2011: 6), today within the area of chemical industry (Gasfabrik, GF). It was excavated in several campaigns during the following 100 years. The unfortified proto-urban site was inhabited between 150 and 80 BCE (La Tène D1) as indicated for example by fibula types and coins. It comprised houses, gardens and pens as well as craft zones, and two cemeteries. The majority of archaeological and faunal remains stem from the c. 585 pits which had been filled with "waste" after they had become unusable as e.g. storage cellars (Hecht and Niederhäuser, 2011: 47). Five horse limb bones and one tooth were chosen for the analysis based on calculability of the horses' withers height (except for the tooth).

2.1.3. Aventicum

Avenches, canton Vaud, was once the capital of the Helvetii known as Aventicum (Castella and Meylan, 2008). Celtic temples had been built in the first century BC, and under the reign of the Roman Emperor Augustus, who forced the urbanisation of the Celtic provinces, Roman town structures were added in the first decade CE which persisted about three hundred years (Blanc and Frei-Stolba, 2001). Aventicum became a Roman colony in 71 CE and was incorporated into *Germania Superior* when the province was formed in 85 CE (Blanc et al., 2001).The three radii studied here stem from sacrificial pits nearby the temples *au Lavoëx* and were chosen due to their large size (Deschler-Erb, 2015).

2.2. Modern horse populations

The matrilineages of the ancient horses were compared to modern horse mtDNA variability. The comparison of modern animal sequences to ancient genetic data has to be treated with caution. In ancient times, there were no breeds in today's sense (Johnstone, 2004: 88f) yet different morphotypes were described, for example by Vegetius (mulom. 3,6,2: Lommatzsch, 1903). We selected breeds that come from regions belonging to the Late Roman Republic in c. 100 BCE (Crook et al., 1994: add. mat. 1), contemporaneous to the occupation of both Mormont and Basel-GF. They were grouped into: Spain (*Hispania Citerior* and *Ulterior*); Italy (*Italia*, *Sardinia*, *Gallia Cisalpina*), and Croatia (*Illyricum*). In addition, breeds from Switzerland, southern Germany and eastern France represent the home region of the Celtic horses investigated here. Breeds were chosen due to their "indigenous" status according to the Domestic Animal Diversity Information System (DAD-IS; dad.fao.org), to availability on GenBank (ncbi.nlm.nih.gov) in April 2015 and when the authors sampled material from maternally unrelated individuals. As Celtic horses are ponies by today's standards (withers heights <148 cm) (Fédération Equestre Internationale, 2015: 60) we compared them also to European pony breeds from Spain, Italy, the Carpathians, European Russia, and Northern Europe (including the British Isles). In total, we assembled sequences from 35 museum and 726 modern specimens representing 30 breeds (Table S1).

2.3. Metrics

Measurements of greatest length (GL) and smallest breadth of diaphysis (SD) of metacarpi and/or radii from Mormont were taken from Méniel (2014: 263f); and from Aventicum and Basel-GF measured according to von den Driesch (1976). Withers heights were calculated from GL of all bones according to May (1985); note that this results in an estimation disregarding sexual dimorphism or robustness.

$$\text{Metacarpus}: \quad \text{GL}\ (\text{mm}) * 0.6102 = \text{WH}\ (\text{cm})$$

$$\text{Radius}: \quad \text{GL}\ (\text{mm}) * 0.4111 = \text{WH}\ (\text{cm})$$

2.4. Sample preparation, DNA extraction and PCR analysis

Sample preparation and DNA extraction were performed as previously described in Elsner et al. (2014) following the User Developed Protocol: „Purification of total DNA from compact animal bone using the DNeasy® Blood & Tissue Kit "(Qiagen, Basel, Switzerland) in dedicated aDNA facilities physically separated from post-PCR laboratories. Standard precautions and measures in aDNA processing were adhered to (Shapiro and Hofreiter, 2012).

PCR for mtDNA and the nuclear (nc) loci (grey, *AMELX/Y*, *ZFX/Y*) was set up in 25 μl volumes containing 1.5 U AmpliTaq Gold, 1× GeneAmp 10× PCR Gold Buffer (150 mM Tris-HCl, 500 mM KCl, pH 8.0) and 2 mM $MgCl_2$ (all Applied Biosystems, Hombrechtikon, Switzerland); 0.4 mM dNTP Mix (Promega, Dübendorf, Switzerland); 0.2 μM of each primer; 20 μg/μl BSA (bovine serum albumin, Roche, Basel, Switzerland), and up to 10 μl template DNA on a Mastercycler ProS (Eppendorf, Allschwil, Switzerland). The cycling conditions were: 12 min initial denaturation, followed by either 50 (for mtDNA) or 70 (for ncDNA) cycles of denaturation at 95 °C for 40 s, annealing at 52 °C to 58 °C (see Tables 2–4) for 30 s, and extension at 72 °C for 30 s, with a final extension of 60 s at 72 °C. Non-template controls were performed alongside all amplifications. Amplification products were visualised on 2% agarose gels. To authenticate sequence patterns and to exclude potential contamination, at least two PCR products from two independent extractions were obtained.

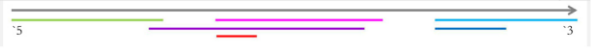
PCR products of mtDNA amplifications were cloned with the TOPO TA Cloning Kit (Invitrogen, Zug, Switzerland) following the manufacturer's protocol, except that the reaction volume was halved. Two clones of each PCR product were Sanger sequenced by Microsynth (Balgach, Switzerland). The products of the ncDNA PCR were premixed with tailed sequencing primers (Binladen et al., 2007) and also Sanger sequenced by Microsynth.

The coat colour SNPs were amplified in multiplex PCR; the first step in 20 μl volumes containing 2.5 U AmpliTaq Gold, 1× GeneAmp 10× Gold Buffer, and 4 mM $MgCl_2$, 0.1 μM of each primer, 20 μg/μl BSA, and 4 μl template DNA. After 30 cycles of amplification as described above, PCR products were diluted 1:20; 5 μl were used in the second, singleplex amplification step (40 cycles) including 0.5 U AmpliTaq Gold, 1× GeneAmp 10× Gold Buffer, and 4 mM $MgCl_2$, 0.2 μM of each primer, and 20 μg/μl BSA. Again, non-template controls were performed and PCR products visualised on 2% agarose gels.

Pyrosequencing of coat colour SNPs of the Basel-GF and Aventicum samples was performed in the Institute for Animal Sciences, Humboldt University Berlin and of Mormont specimens at the Life Science Training Facilities in the Biozentrum, University of Basel according to manufacturer's instructions and Ludwig et al. (2009) on PSQ™ 96MA (Biotage, Uppsala, Sweden).

Table 2
Primer sequences for mitochondrial haplotyping, and amplification strategy. Target lengths are given excluding primers. Reference sequence from Xu and Arnason (1994).

Name	Sequence 5′-3′	Position of target in reference sequence NC_001640	Annealing temperature (°C)	Length of target (bp, colour)
Ec1_f Ec1_r	TTCTTCCCCTAAACGACAACA GACGTACATAGGCCATTCATAAGA	15492-15563	52	72 (green)
Ec4_f Ec4_r	GAATGGCCTATGTACGTCGTG GACTTGGATGGGGTATGCAC	15591-15669	55	79 (4f+4r, pink)
Ec4b_r	ATATTATGTACATGCTTATTATTCA	15591-15609	50	19 (4f+4br, red)
Ec2_f Ec2_r	ACATAACACCATACCCACCTGACA GATGGGGTATGCACGATCAATAAT	15557-15659	55	103 (violet)
Ec5_f Ec5_r	ACCCCATCCAAGTCAAATCA TAGTTGGGAGGGTTGCTG	15696-15758	55	63 (5f+5r, light blue)
Eca1_r	GGCTTGGTGATTAAGCTCGT	15696-15730	52	35 (5f+a1r, blue)



2.5. Mitochondrial haplotyping

To investigate the matrilineages six partially overlapping and interspersed targets of the mtDNA d-loop with different lengths covering nucleotide positions 15,492–15,669 and 15,696–15,758 (Table 2) (Xu and Arnason, 1994) were amplified. Primers were taken and/or modified from Weber (2005), Cieslak et al. (2010) and Elsner et al. (2014) except for Ec4b_r (this study).

2.6. Molecular sex identification

For the molecular sex identification primer sets Ecab_AMELX/Y from Lippold et al. (2011a) and ZFX/Y_Equus (this study, Table 3) were applied.

Table 3
Primer sequences to identify the molecular sex of horse remains. Reference sequences from Han et al. (2010).

Name	Sequence 5′–3′	Reference sequence	Position of target in reference sequence	Annealing temperature (°C)	Length of target (bp)
ZFX_Equus_F	ATTATATCTGGCCCAGGACT	DQ179230	326–382	55	58
ZFX_Equus_R	TGCCTAGCTTCCAAATCTAA				
ZFY_Equus_F	GAATTTCCTACATGCCCATA	DQ179229	214–263	55	50
ZFY_Equus_R	ATAAAGTCATGAGCCGGATA				

2.7. Coat colour analysis

The investigation of nuclear SNPs in the genes associated with coat colour phenotypes was performed as described in Ludwig et al. (2009). Samples were tested for the basic colours bay, chestnut and black via the *MC1R*- and *ASIP*-loci (Marklund et al., 1996; Rieder et al., 2001). Epistatic dilutions Cream (Mariat et al., 2003) and Silver (Reissmann et al., 2007) and spottings Overo (Santschi et al., 1998), Tobiano (Brooks et al., 2002) and Sabino (Brooks and Bailey, 2005) were examined. In addition, the grey-locus, resulting in a complete white coat in adult horses regardless of original colour, was investigated. This phenotype is caused by a duplication in intron 6 of the *STX17* gene (Rosengren Pielberg et al., 2008). We designed primer pairs to target short fragments of c. 100 bp, first, for the region that spans the end and the beginning of the 4.6 kb duplication (Table 4, red) to detect the mutated allele as well as second, a primer pair to target the end of the fragment (Table 4, blue) which would be present in both wildtype (not grey) and mutated genotype (grey). The suitability of the primer systems was tested on five randomly chosen Freiberger (bay or chestnut) and Camargue (grey) horses each (Fig. S1) at the Institute of Genetics, University of Bern.

2.8. Sequence data analysis

D-loop and *STX17* sequences were examined with BioEdit (Hall, 1999). Mitochondrial haplotypes were determined by comparison with the reference sequence NC_001640 (Xu and Arnason, 1994). We used the nomenclature of Cieslak et al. (2010) to determine the haplotype (Table S2). Haplotype and nucleotide diversity as well as pairwise difference F_{ST} values were computed with Arlequin 3.5 (Excoffier and Lischer, 2010), excluding five ancient samples with missing data (see Table 5). The comparison of Iron Age and modern sequences was done on the basis of haplotype frequencies and pairwise differences for each breed, geographic origin and type (horse, pony). Sequences were pruned to positions 15,494–15,740 to include as many samples as possible. F_{ST} values were interpreted as follows: values from 0 to 0.05 indicate non to little genetic differentiation, 0.05–0.15 moderate differentiation, 0.15–0.25 large differentiation, and values above 0.25 very large differentiation. Negative F_{ST} values equate to zero (Hartl and Clark, 2007: 288). Based on relative haplotype frequencies, Principal Component Analysis (PCA) was computed with PAST (Hammer et al., 2001). Singleton haplotypes were removed from this analysis. Median Joining Networks (MJN) (Bandelt et al., 1999) were constructed with Network 4.6.1.2 (fluxus-engeneering.com) using the following parameters: insertions and deletions were double weighted (default 50), transition: transversion ratio was set to 1:10, and variable sites were down weighted according to the number of deviations from the majority at each nucleotide position excluding haplotype defining sites (Tables S2 & S3). The dataset was reduced to 360 modern (one representative per haplotype per breed) and 14 ancient sequences. The probabilities P of allelic dropout for the SNP loci were calculated as in Gagneux et al. (1997): $P = K * (K/2)^{n-1}$, where K is the number of observed allelic dropouts divided by the number of all positive amplifications of heterozygous individuals, and n is the number of PCR replications.

Table 4
Primer sequences to detect i) the duplication causing premature greying (grey-locus) and ii) the wildtype (no duplication). Figure shows position of target sequences. Reference sequences from Rosengren Pielberg et al. (2008).

Name	Sequence 5'-3'	Position of targetin reference sequence EU606026	EU606027	Annealing temperature (°C)	Length of target (bp, colour)
Both_f	GCACCACCTGGGAACTCA				
Both_r	CTGGAGTGTGCACCAGAATC	5830-5900	10407-10477	52	71 (blue)
Dup_r	GAGAAGTTGGGCAAGAGCAG	-	5830-5889	58	61 (red)

3. Results

3.1. Metrics

The measurements of SD and GL as well as the estimation of withers height of the La Tène and Roman time horses revealed variation at all archaeological sites. Furthermore, based on metacarpi the individuals cluster into a smaller and into a larger group, based on the radii into three groups (Fig. 2). Note that not all individuals are represented by both skeletal elements. There is a linear correlation between length and breadth ($R^2 = 0.81$ for metacarpi, $R^2 = 0.9$ for radii) indicating isometric growth and no robustness differences.

The archaeozoological defined standard withers height of Celtic horses ranges between c. 110 cm to 130 cm (Peters, 1998; Arbogast et al., 2002); the majority of animals investigated here accord to this (Fig. 3). Five horses were larger than 130 cm: three from the Late Iron

Table 5
Differences to the reference sequence NC_001640 between nucleotide positions 15,492–15,669 and 15,696–15,758. Samples indicated by an asterisk have missing data at nucleotide positions: MO10, MO14, MO18: 15,564–15,669; BGF2, AV3: 15,564–15,690 and 15,610–15,669. Cieslak et al. (2010) disregard hotspot nucleotide positions 15,585; 15,597; 15,604 and 15,650 for haplotype assignment, they are mentioned here for completeness.

Sample	Differences to NC_001640 (Xu and Arnason, 1994) 15,...	Haplotype after Cieslak et al. (2010)
MO1	495C; 538G; 602T; 650G; 709T; 720A	I
MO2	495C; 650G; 666A; 720A	D3
MO4	495C	D2
MO5	494C; 495C; 496G; 534T; 585A; 603C; 649G; 720A	X2b
MO6	495C; 602T; 617C; 659C; 720A	B1
MO9	495C; 542T; 585A; 597G; 602T; 615G; 635T; 650G; 666A; 703C; 720A	X3c1 + 615
MO10*	495C; 720A	Haplogroup A/B/C/D/F?
MO12	495C; 585A; 601C; 602T; 720A	F
MO14*	495C; 546T; 703C; 720A	Haplogroup K?
MO15	494C; 495C; 496G; 534T; 603C; 649G; 720A	X2b
MO16	495C; 585A; 601C; 602T; 720A	F
MO17	494C; 495C; 496G; 534T; 603C; 649G; 720A	X2b
MO18*	494C; 495C; 496G; 534T; 720A	Haplogroup X2
BGF1	495C; 585A; 601C; 602T; 720A	F
BGF2*	494C; 495C; 496G; 534T; 603C; 649N; 720A	X2b
BGF3	495C; 542T; 597G; 602T; 631G; 635T; 650G; 666A; 703C; 720A	X3c1 + 631
BGF5	495C; 585A; 601C; 602T; 720A	F
AV2	495C; 542T; 585A; 597G; 602T; 615G; 635T; 650G; 666N; 703C; 720A	X3c1 + 615
AV3*	494C; 495C; 496G; 534T; 602T; 603C; 604A; 720A	Haplogroup X2

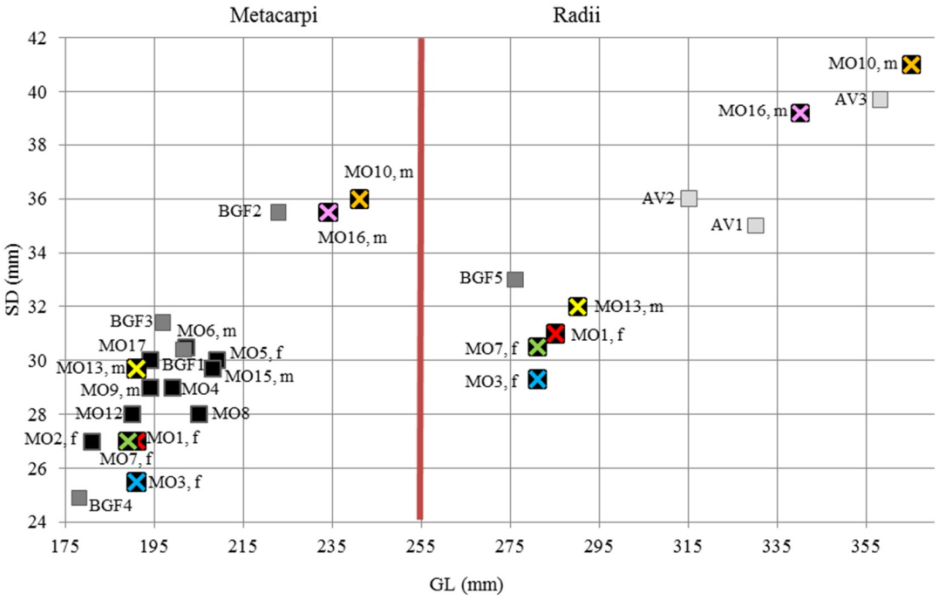


Fig. 2. Greatest length (GL) against smallest breadth of diaphysis (SD) of metacarpi (left panel) and radii (right panel). Bones from Mormont (black), Basel-GF (grey) and Aventicum (light grey). Mormont individuals with data from both skeletal elements indicated by symbol; morphological and/or molecular sex determination: f = female, m = male.

Age: two from Mormont (MO10: 149 cm, MO16: 142 cm) and one from Basel-GF (BGF2: 136 cm), and both Roman time horses (AV1: 136 cm, AV3: 147 cm) from Aventicum. The average withers height of the Mormont horses was, based on 15 measurable metacarpi and six radii, 124 cm (119 cm without the two large animals). The average in Basel-GF was 120 cm and in Aventicum 137 cm (141 cm for the Roman time individuals).

3.2. Mitochondrial haplotyping

In Mormont ten metacarpi were genetically well preserved, three only allowed for a partial amplification of the target region, and five had no amplifiable mtDNA preserved. It was possible to amplify mtDNA from four out of six samples from Basel-GF and from two out of three radii (one Iron Age, one Roman) from Aventicum. This results in an overall amplification success of 56% (19/34).

We identified eleven haplotypes belonging to six haplogroups (hg). Most prevalent is hg X2 (n = 6), followed by hg F (n = 4), hg X3 (n = 3), hg D (n = 2), hg B (n = 1) and hg I (n = 1). Additionally, one sample each might be associated with hg K and hgs A/B/C/D/F, respectively (Table 5). Haplotype sharing within and between archaeological sites was observed: haplotype F is shared by MO12, MO16, BGF1 and BGF5. MO15 and MO17 have the same haplotype X2b, MO5 and BGF2 also share variants of X2b, and despite some missing data MO18 and AV3 can be assigned to haplogroup X2 as well. MO9, BGF3 and AV2 possess variants of haplotype X3c1, one of which previously undescribed (BGF3). Horses with withers height below 130 cm carried haplotypes B1, D2, D3, F, I, X2b, and X3c1; they share haplotype F and haplogroup X2 with the large horses. Haplotype (0.92 ± 0.06) and nucleotide (0.3 ± 0.02) diversity is very high in the investigated Iron Age horses (Table S4). This result is mirrored by the horses from Mormont: haplotype diversity = 0.97 ± 0.06, nucleotide diversity5 = 0.3 ± 0.02.

3.3. Molecular sex identification

Molecular sex could be identified for five specimens from Mormont, the other samples were not preserved well enough to enable the amplification of targets in the amelogenin and/or zinc-finger genes (Table 6). This confirms the results of the morphological sex determination for MO5 and MO16; MO2, MO6 and MO9 were previously undetermined.

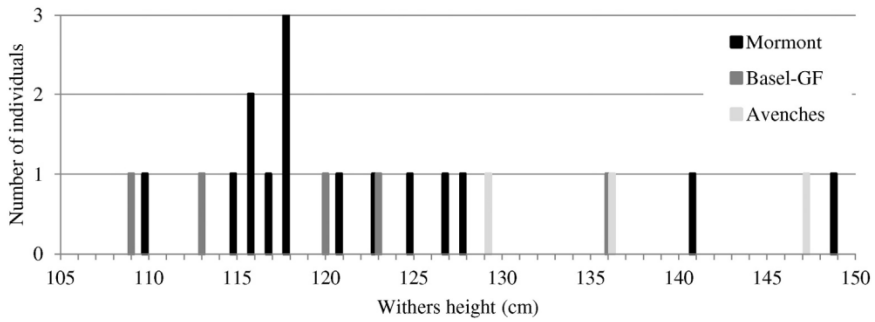


Fig. 3. Estimated withers height (May, 1985) in cm from metacarpi and/or radii from Mormont (black), Basel-GF (grey) and Aventicum (light grey). When measurements of both bones were available, the mean was used.

Table 6
Results for molecular sex identification with *AMELX/Y* and *ZFX/Y*.

Sample	AMELX	AMELY	ZFX	ZFY	Sex
MO2	1/3	0/3	1/2	0/3	Female
MO5	1/3	0/2	2/2	0/4	Female
MO6	0/2	1/2	2/2	1/3	Male
MO9	0/2	1/1	2/2	2/3	Male
MO16	0/1	0/1	2/2	2/2	Male

3.4. Coat colour

For six of the Mormont and two of the Basel-GF samples DNA preservation allowed for the amplification of SNPs in genes associated with coat colour (Table 7). The specimens from Aventicum were not preserved well enough for SNP typing. All positive samples revealed basic colourations. One large horse (MO16) was bay, of the smaller horses (<130 cm) three had a black coat and four were chestnut. We did not detect any mutations in the dilution and spotting loci. The Mormont specimens were also tested for the duplication that causes premature greying. For five individuals the target at the end of the 4.6 kb fragment present in both wildtype and mutated genotype could be amplified twice (once for MO6, see Table 7), but the shorter PCR products for the grey-locus were negative four times for each individual suggesting all tested animals were not grey. The probability of allelic dropout for the only heterozygous SNP, *MC1R*, was $p = 0.0015$ with four PCR replications.

3.5. Comparison with modern horses

The Median Joining Network shows the widespread distribution of Late Iron Age horse mtDNA d-loop sequences within the variation of southern European horse and European pony breeds (Fig. 4). The ancient horses are represented in the major haplogroups (B, D, F, I, X2, X3) except for hg K (see above).

The fixation index based on the number of pairwise differences between each breed and the ancient horses indicated substantial structuring ($F_{ST} = 0.12$, $p < 0.001$) (see Table S5 for pairwise population F_{ST}). The genetic differentiation between the geographic regions is moderate ($F_{ST} = 0.06$, $p < 0.001$). F_{ST}-values for the regional groups indicate close relationships between all European horse and pony breeds and the Celtic horses, except for the Spanish breeds which are moderately different (Table S6). This outcome is supported by PCA based on the frequencies of haplotypes with loadings >10% (Fig. 5; frequencies of all haplotypes in Fig. S2). The Iron Age horses stand out from the modern breeds because of their unique variability but tend to be closer to the northern European ponies which share all haplotypes present in the ancient dataset.

4. Discussion

This study provides first insight into mtDNA d-loop diversity, molecular sex and coat colour of Late La Tène horses in Switzerland. DNA preservation was as expected for the region and time frame explored, however, the results for the nuclear markers were better for the recently excavated site on the hill of Mormont (Pruvost et al., 2007; Bollongino et al., 2008). The withers height of the investigated horses fell mostly within the variation of standard Iron Age horses, yet five individuals were larger than 130 cm reaching up to 149 cm. Two of these were identified as male, but smaller stallions were also present, thus sexual dimorphism does not explain the height differences. Moreover, the relation between length and breadth (robustness) is linear, indicating that smaller and larger horses did not belong to different morphotypes (Brooks et al., 2010; Dzierzecka and Komosa, 2013). Only one horse exceeded the defined pony range of maximal 148 cm. The investigation of the maternal lineages revealed high nucleotide and haplotype diversity within the upper range of variance in modern European breeds (see Table S4). The horses belonged to six haplogroups (B, D, F, I, X2, X3) which are rare in wild horses (Weinstock et al., 2005; Cieslak et al., 2010; Lorenzen et al., 2011; Orlando et al., 2013). They were first described from domestic horses from sites in Novosibirsk, Russia, dating to c. 2000 BCE, and Moldova and Romania around 1250 BCE (Cieslak et al., 2010). A haplogroup-package dominated by hgs D, K, X2 and X3 is subsequently found in archaeologic sites throughout Eurasia (Vila et al., 2001; Di Bernardo et al., 2004; Keyser-Tracqui et al., 2005; McGahern et al., 2006; Cai et al., 2007; Cai et al., 2009; Lira et al., 2010; Cieslak et al., 2010; Priskin et al., 2010; Bower et al., 2013).

The large matrilineage diversity is striking, particularly given that the majority of horses, namely those from Mormont, belonged to a potentially contemporaneous local population which should include directly related individuals. The occurrence of shared lineages in the sites Mormont and Basel-GF might indicate horse exchange between Helvetii and Rauricii. The ancient matrilineages show affinities to modern horse and pony breeds from most regions investigated. They tend to have bequeathed more to northern European and British Isles pony breeds and less to Spanish breeds. However, considering that domestic horses were first introduced to Switzerland in the Late Neolithic or Early Bronze Age, i.e. around 2500–2000 BCE (Schibler and Studer, 1998: 177), it is remarkable that most major haplogroups are present in the Iron Age archaeological record, notably in view of the rareness of archaeologic horse remains.

With morphological and molecular methods, five mares and seven stallions were identified, all from the hill site of Mormont. This corresponds to the male:female ratio of 1:1.5 amongst all morphologically sex determined horses in Mormont (Méniel, 2014: 17).

Coat colouration seems to be rather uniform. We would expect more variety: Ludwig et al. (2009) documented a rapid and substantial increase in coat colourations already in the Bronze Age, at least in Asia

Table 7
Results from the coat colour analysis. In addition to the phenotype, the genotypes for the eight SNPs investigated are shown; differences from the wildtype are indicated in bold and only detected at the *ASIP*- and *MCR1*-loci. The duplication causing premature greying was not detected in any of the Mormont samples (Avenches and Basel-GF samples were not tested). Unreproducible results are parenthesised.

Sample	Phenotype	Nuclear Genes								
		ASIP	*MC1R*	*EDNRB* Overo spotting	*KIT13* Tobiano spotting	*KIT16* Sabino spotting	*MATP* Cream dilution	*SILV9* Silver dilution	*SILV11* Silver dilution	GREY
MO4	(Chestnut)	**(a/a)**	**(e/e)**	ov/ov	KM0/KM0	(sb1/sb1)	C/C	(z/z)	z/z	–
MO5	Black	**a/a**	**E/e**	ov/ov	KM0/KM0	sb1/sb1	C/C	z/z	z/z	No
MO6	Black	**a/a**	**E/e**	ov/ov	KM0/KM0	sb1/sb1	C/C	z/z	z/z	(No)
MO9	Black	**a/a**	**E/e**	ov/ov	KM0/KM0	sb1/sb1	C/C	z/z	z/z	No
MO16	Bay	A/A	E/E	ov/ov	KM0/KM0	sb1/sb1	C/C	z/z	z/z	No
MO17	Chestnut	**a/a**	**e/e**	ov/ov	KM0/KM0	sb1/sb1	C/C	z/z	(z/z)	No
BGF1	Chestnut	**a/a**	**e/e**	ov/ov	KM0/KM0	sb1/sb1	C/C	(z/z)	z/z	–
BGF4	Chestnut	–	**e/e**	–	KM0/KM0	–	C/C	z/z	z/z	–

Differences from the wildtype are indicated in bold; the wildtype is given in capital letters in the genetic nomenclature.

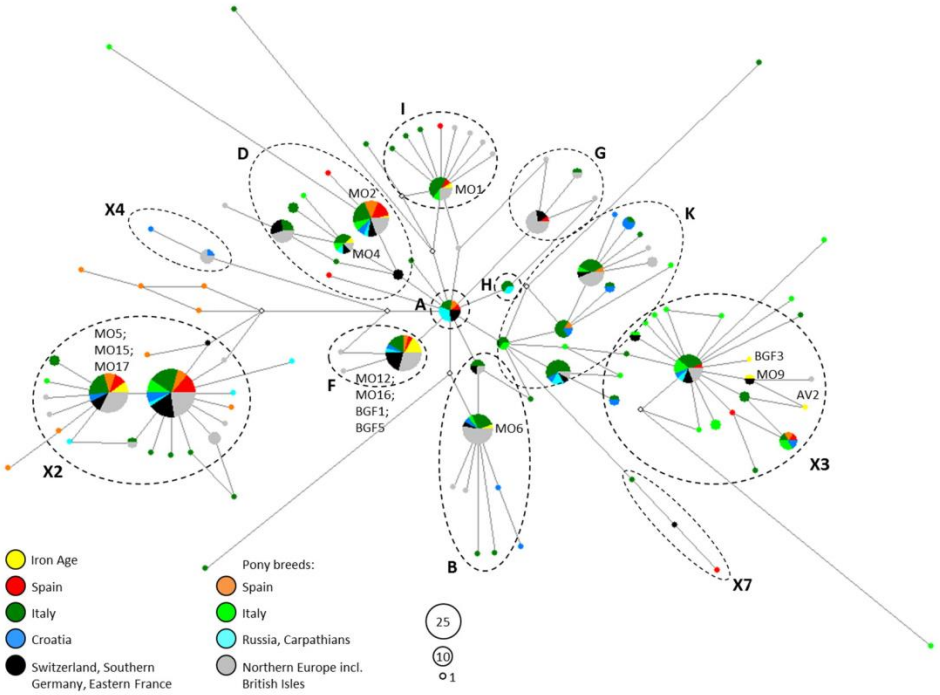


Fig. 4. Median Joining Network of 14 ancient and 355 modern mtDNA d-loop sequences. Circle size is proportional to haplotype frequency. Nomenclature follows Cieslak et al. (2010).

and eastern Europe, and in northern Europe spotted Iron Age horses were detected (Svensson et al., 2012) in comparable datasets. However, monochrome horses were favoured over skewbalds and white markings on face and leg according to written Roman sources (e. g. Corp. Hippiatr. Gr., 1, 115: Oder and Hoppe, 1924–1927; Geop. 16,2: Wappmann, 1985). Breeders probably had noticed hearing, visual, and neurological impairment and even stillbirths coming along predominantly with spottings, especially in homozygous individuals

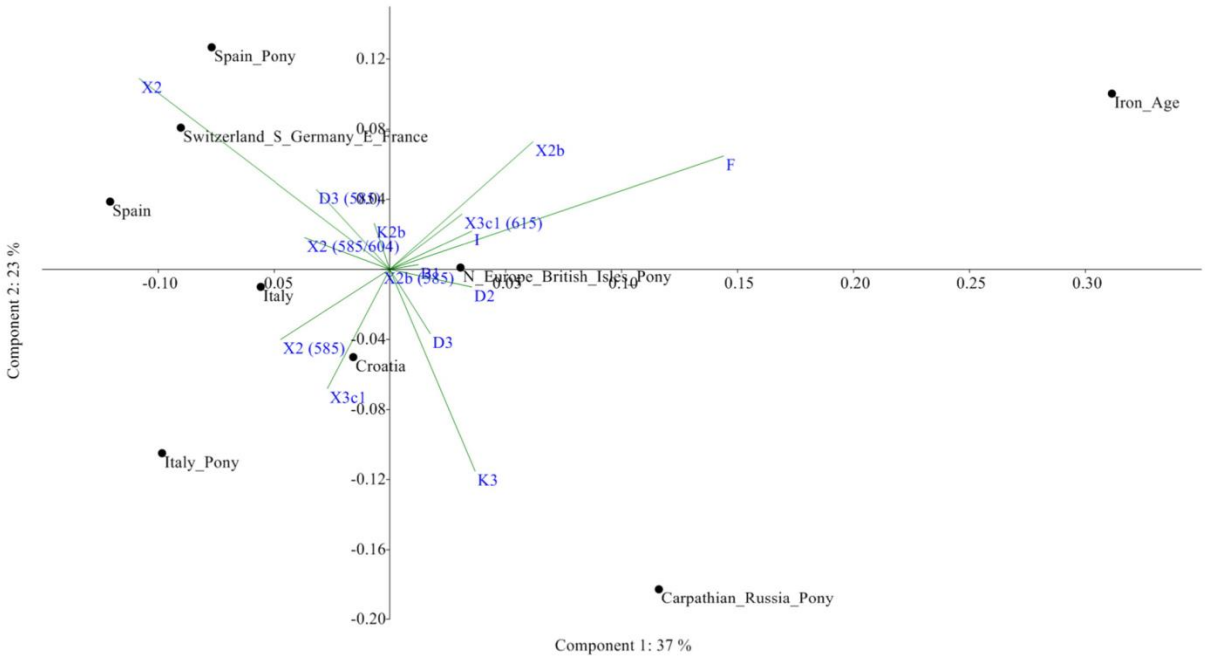


Fig. 5. PCA plot based on relative mtDNA haplotype frequencies of the haplotypes present in the Iron Age horses and additional haplotypes with loadings over 10% (see Fig. S2). The two dimensions display 60% of the total variance.

(Reissmann, 2009: 20; Bellone, 2010) and thus may have selected against those phenotypes. Notably premature greying resulting in a white coat regardless of original colour was also not detected. Again, we expected a different result, as white horses played an important role in Roman and also in Germanic ceremonies (Tacitus, Germ. 1,10: Kretschmer, 1986; Johnstone, 2004: 61). However, of the horses investigated, only the specimens from Aventicum stem from an unambiguous ritual context and those were not preserved well enough for genetic coat colour identification. Assuming that ceremonial activities were also performed on the hill of Mormont, we find no evidence that specially coloured horses were preferred in ritual, which apparently also applies for sex as both females and males were present.

Some important archaeozoological questions concerning horse exchange, breeding aims and selection criteria e.g. for ceremony have to remain open yet, due to both the small sample size and insufficient data for comparison. Further complicating is the fact that Roman horses, too, were not standardised and the workaday animals were usually smaller than military or circus breedings (Junkelmann, 1990: 39). It appears that mtDNA d-loop variation offers too little structuring, but because it is highly conservative the investigation of the paternal inherited Y-chromosome cannot contribute to the question of horse provenience either (Lippold et al., 2011a; Wallner et al., 2013). The exploration of nuclear markers beyond sex and colouration (Schubert et al., 2014) might give insight into possibly distinct characteristics of horses bred by Celtic and Roman stud farmers. Further approaches like stable isotope analysis, particularly of strontium (Slovak and Paytan, 2011), seem even more promising to solve these important issues. It might thus be necessary to dig deeper into the past and to include archaeological material from both Rome and its provinces, and regions outside the Roman Empire to trace the origin and breeding history of small and large horses.

5. Conclusion

The horse remains excavated from the archaeological sites Mormont, Basel-GF and Aventicum, dating to 150–50 BCE (La Tène D) and to c. 100 CE, revealed a high diversity of matrilineages. The most frequent haplogroups F and X2 were shared between the sites, and between small and large horses. While all determinable large individuals (>140 cm withers height) were male, both sexes were present amongst horses with withers heights between 110 and 130 cm. The detection of solely monochrome coats and the absence of white colourations might indicate selection. The simplified approach to detect premature greying developed in this study provides a relevant tool for aDNA research concerning the phenotypes of (pre)historic horses. Compared to modern indigenous breeds, Late Iron Age horses seem to have bequeathed more to northern European ponies than to Spanish breeds. By broadening the methodological spectrum and by complementing the dataset both spatially and temporally, we hope to address archaeozoological questions on horse exchange, breeding aims, and selection criteria e.g. in ritual contexts more comprehensively.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jasrep.2016.03.007.

Acknowledgements

Samples were kindly provided by Archéologie Vaudoise (Mormont), Site et Musée Romain d'Avenches (Aventicum) and Archäologische Bodenforschung Basel-Stadt (Basel-Gasfabrik). The authors thank Tosso Leeb and Michaela Drögemüller (Institute for Genetics, University of Bern), Andreas Papassotiropoulos and Kim Dung Huynh (Biozentrum, University of Basel), Arne Ludwig (Institute for Zoo- and Wildlife Research) and Mélanie Pruvost (then Institute for Animal Sciences, Humboldt University Berlin), Patrice Méniel and Pauline Nuviala (University of Burgundy, Dijon), co-editor Andy Howard, University of Durham, and one anonymous reviewer. Julia Elsner was funded by Swiss National Science Foundation (K-31K1_120528/1), Freiwillige Akademische Gesellschaft Basel, Nikolaus und Bertha Burckhardt-Bürgin-Foundation.

References

Achilli, A., Olivieri, A., Soares, P., Lancioni, H., Hooshiar Kashani, B., Perego, U.A., Nergadze, S.G., Carossa, V., Santagostino, M., Capomaccio, S., Felicetti, M., Al-Achkar, W., Penedo, M.C., Verini-Supplizi, A., Houshmand, M., Woodward, S.R., Semino, O., Silvestrelli, M., Giulotto, E., Pereira, L., Bandelt, H.J., Torroni, A., 2012. Mitochondrial genomes from modern horses reveal the major haplogroups that underwent domestication. PNAS 109, 2449–2454. http://dx.doi.org/10.1073/pnas.1111637109.

Arbogast, R.M., Benoit, C., Lepetz, S., Méniel, P., Yvinec, J.-H., 2002. Archéologie du cheval. Des origines à la période moderne en France. Hespérides, Paris.

Bandelt, H.J., Forster, P., Rohl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16, 37–48.

Bellone, R.R., 2010. Pleiotropic effects of pigmentation genes in horses. Anim. Genet. 41, 100–110. http://dx.doi.org/10.1111/j.1365-2052.2010.02116.x.

Binladen, J., Gilbert, M.T., Campos, P.F., Willerslev, E., 2007. 5′-tailed sequencing primers improve sequencing quality of PCR products. Biotechniques 42, 174–176. http://dx.doi.org/10.2144/000112316.

Blanc, P., Frei-Stolba, R., 2001. Die Stadtentwicklung. Archäol. Schweiz. 24. http://dx.doi.org/10.5169/seals-18959.

Blanc, P., Frey-Kupper, S., Frei-Stolba, R., 2001. Die Anfänge der Hauptstadt der Helvetier. Archäol. Schweiz. 24. http://dx.doi.org/10.5169/seals-18956.

Boessneck, J., von den Driesch, A., Meyer-Lemppenau, U., Wechsler-von Ohlen, E., 1971. Die Tierknochenfunde aus dem Oppidum von Manching. Franz Steiner, Wiesbaden.

Bollongino, R., Tresset, A., Vigne, J.D., 2008. Environment and excavation: pre-lab impacts on ancient DNA analyses. C.R. Palevol 7, 91–98. http://dx.doi.org/10.1016/j.crpv.2008.02.002.

Bower, M.A., Edwards, C.J., Evans, C., Downes, C.S., 2013. Horse husbandry in Iron Age and Romano-British East Anglia: ancient DNA analysis of horses at Langdale Hale, Cambridgeshire. In: Evans, C., Appleby, G., Lucy, S., Regan, R. (Eds.), Process and History: Romano-British Communities at Colne Fen, Earith: An Inland Port and Supply Farm. University Press, Cambridge, pp. 140–143.

Brem, H., Haldimann, M.-A., 1999. Handel und Verkehr: Die Latènezeit. In: Müller, F., Kaenel, G., Lüscher, G. (Eds.), SPM IV: Eisenzeit. Verlag Schweizerische Gesellschaft für Ur- und Frühgeschichte, Basel, pp. 215–219.

Brooks, S.A., Bailey, E., 2005. Exon skipping in the KIT gene causes a Sabino spotting pattern in horses. Mamm. Genome 16, 893–902. http://dx.doi.org/10.1007/s00335-005-2472-y.

Brooks, S.A., Makvandi-Nejad, S., Chu, E., Allen, J.J., Streeter, C., Gu, E., McCleery, B., Murphy, B.A., Bellone, R., Sutter, N.B., 2010. Morphological variation in the horse: defining complex traits of body size and shape. Anim. Genet. 41, 159–165. http://dx.doi.org/10.1111/j.1365-2052.2010.02127.x.

Brooks, S.A., Terry, R.B., Bailey, E., 2002. A PCR-RFLP for KIT associated with tobiano spotting pattern in horses. Anim. Genet. 33, 301–303. http://dx.doi.org/10.1046/j.1365-2052.2002.00854.x.

Brunetti, C., Kaenel, G., Méniel, P. (Eds.), 2014. Les Helvètes au Mormont. Une énigme dans le monde celtique. SAS Archeodunum, Chaponnay.

Cai, D.W., Han, L., Xie, C.Z., Li, S.N., Zhou, H., Zhu, H., 2007. Mitochondrial DNA analysis of Bronze Age horses recovered from Chifeng region, Inner Mongolia, China. Prog. Nat. Sci. 17, 544–550.

Cai, D.W., Tang, Z.W., Han, L., Speller, C.F., Yang, D.Y.Y., Ma, X.L., Cao, J.E., Zhu, H., Zhou, H., 2009. Ancient DNA provides new insights into the origin of the Chinese domestic horse. J. Archaeol. Sci. 36, 835–842. http://dx.doi.org/10.1016/j.jas.2008.11.006.

Castella, D., Meylan, M.F., 2008. Topographie sacrée et rituels: le cas d'Aventicum, capital des Helvètes: Actes du colloque international d'Avenches, 2–4 Novembre 2006: Antiqua.

Cieslak, M., Pruvost, M., Benecke, N., Hofreiter, M., Morales, A., Reissmann, M., Ludwig, A., 2010. Origin and history of mitochondrial DNA lineages in domestic horses. PLoS One 5, e15311. http://dx.doi.org/10.1371/journal.pone.0015311.

Cieslak, M., Reissmann, M., Hofreiter, M., Ludwig, A., 2011. Colours of domestication. Biol. Rev. Camb. Philos. Soc. 86, 885–899. http://dx.doi.org/10.1111/j.1469-185X.2011.00177.x.

Crook, J.A., Lintott, A., Rawson, E. (Eds.), 1994. The Cambridge Ancient History Volume 9: The Last Age of the Roman Republic, 146–43 BC. University Press, Cambridge.

Deschler-Erb, S., 2015. Tier und Kult - Spezielle Tierknochendeponierungen der Spätlatène- und Römerzeit aus Aventicum/Avenches (CH) im nordalpinen Vergleich. Musée Romain d'Avenches, Avenches.

Di Bernardo, G., Galderisi, U., Del Gaudio, S., D'Aniello, A., Lanave, C., De Robertis, M.T., Cascino, A., Cipollaro, M., 2004. Genetic characterization of Pompeii and Herculaneum Equidae buried by Vesuvius in 79 AD. J. Cell. Physiol. 199, 200–205. http://dx.doi.org/10.1002/jcp.10461.

Dietrich, E., Kaenel, G., Weidmann, D., Jud, P., Méniel, P., Moinat, P., 2007. Le sanctuaire helvète du Mormont. Archäol. Schweiz. 30, 2–13. http://dx.doi.org/10.5169/seals-109261.

Dzierzecka, M., Komosa, M., 2013. Variability of the proximal phalanx in warmblood and coldblood horses - morphological and structural analyses. Belg. J. Zool. 143, 119–130.

Elsner, J., Schibler, J., Hofreiter, M., Schlumbaum, A., 2014. Burial condition is the most important factor for mtDNA PCR amplification success in Palaeolithic equid remains from the Alpine foreland. Archaeol. Anthropol. Sci. 1–11 http://dx.doi.org/10.1007/s12520-014-0213-4.

Excoffier, L., Lischer, H.E., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10, 564–567. http://dx.doi.org/10.1111/j.1755-0998.2010.02847.x.

Fédération Equestre Internationale FEI, 2015. 2015 Veterinary Regulations. Fédération Equestre Internationale, Lausanne.
Gagneux, P., Boesch, C., Woodruff, D.S., 1997. Microsatellite scoring errors associated with noninvasive genotyping based on nuclear DNA amplified from shed hair. Mol. Ecol. 6, 861–868.
Green, M.J., 1992. Animals in Celtic life and Myth. Routledge, London New York.
Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98 (doi: citeulike-article-id:691774).
Hammer, O., Harper, D.A.T., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4, 1–9.
Han, S.-H., Yang, B.-C., Ko, M.-S., Oh, H.-S., Lee, S.-S., 2010. Length difference between equine ZFX and ZFY genes and its application for molecular sex determination. J. Assist. Reprod. Genet. 27, 725–728. http://dx.doi.org/10.1007/s10815-010-9467-7.
Hartl, D.L., Clark, A.G., 2007. Principles of Population Genetics. Sinauer Associates, Inc. Publishers, Sunderland, Mass.
Hecht, Y., Niederhäuser, A., 2011. Alltagskultur und Totenrituale der Kelten. Ein Siedlungszentrum am Oberrhein um 100 v. Chr. - The everyday culture and funerary rituals of the Celts. A central settlement on the Upper Rhine around 100 BC. Christoph Merian Verlag, Basel.
Hyland, A., 1990. *Equus*: The Horse in the Roman World. Yale University Press, New Haven, London.
Iwase, M., Satta, Y., Hirai, Y., Hirai, H., Imai, H., Takahata, N., 2003. The amelogenin loci span an ancient pseudoautosomal boundary in diverse mammalian species. PNAS 100, 5258–5263. http://dx.doi.org/10.1073/pnas.0635848100.
Jansen, T., Forster, P., Levine, M.A., Oelke, H., Hurles, M., Renfrew, C., Weber, J., Olek, K., 2002. Mitochondrial DNA and the origins of the domestic horse. PNAS 99, 10905–10910. http://dx.doi.org/10.1073/pnas.152330099.
Johnstone CJ (2004) A Biometric Study of Equids in the Roman World. (PhD thesis Archaeology department). York: University of York.
Junkelmann, M., 1990. Die Reiter Roms. Teil I: Reise, Jagd, Triumph und Circusrennen. Philipp von Zabern, Mainz.
Kaenel, G., 2014. De quand date l'occupation du Mormont? In: Brunetti, C., Kaenel, G., Méniel, P. (Eds.), Les Helvètes au Mormont Une énigme dans le monde celtique. SAS Archeodunum, Chaponnay, pp. 74–75
Keyser-Tracqui, C., Blandin-Frappin, P., Francfort, H.P., Ricaut, F.X., Lepetz, S., Crubezy, E., Samashev, Z., Ludes, B., 2005. Mitochondrial DNA analysis of horses recovered from a frozen tomb (Berel site, Kazakhstan, 3rd Century BC). Anim. Genet. 36, 203–209. http://dx.doi.org/10.1111/j.1365-2052.2005.01316.x.
Kretschmer, M. (Ed.), 1986. Tacitus: Germania. Verlag Aschendorff, Münster.
Lippold, S., Knapp, M., Kuznetsova, T., Leonard, J.A., Benecke, N., Ludwig, A., Rasmussen, M., Cooper, A., Weinstock, J., Willerslev, E., Shapiro, B., Hofreiter, M., 2011a. Discovery of lost diversity of paternal horse lineages using ancient DNA. Nat. Commun. 2, 450. http://dx.doi.org/10.1038/Ncomms1447.
Lippold, S., Matzke, N.J., Reissmann, M., Hofreiter, M., 2011b. Whole mitochondrial genome sequencing of domestic horses reveals incorporation of extensive wild horse diversity during domestication. BMC Evol. Biol. 11, 328. http://dx.doi.org/10.1186/1471-2148-11-328.
Lira, J., Linderholm, A., Olaria, C., Brandstrom Durling, M., Gilbert, M.T., Ellegren, H., Willerslev, E., Liden, K., Arsuaga, J.L., Gotherstrom, A., 2010. Ancient DNA reveals traces of Iberian Neolithic and Bronze Age lineages in modern Iberian horses. Mol. Ecol. 19, 64–78. http://dx.doi.org/10.1111/j.1365-294X.2009.04430.x.
Lommatzsch, E. (Ed.), 1903. P. Vegeti Renati digestorum artis mulomedicinae libri. B. G. Teubner, Leipzig.
Lorenzen, E.D., Nogues-Bravo, D., Orlando, L., Weinstock, J., Binladen, J., Marske, K.A., Ugan, A., Borregaard, M.K., Gilbert, M.T., Nielsen, R., Ho, S.Y., Goebel, T., Graf, K.E., Byers, D., Stenderup, J.T., Rasmussen, M., Campos, P.F., Leonard, J.A., Koepfli, K.P., Froese, D., Zazula, G., Stafford Jr., T.W., Aaris-Sorensen, K., Batra, P., Haywood, A.M., Singarayer, J.S., Valdes, P.J., Boeskorov, G., Burns, J.A., Davydov, S.P., Haile, J., Jenkins, D.L., Kosintsev, P., Kuznetsova, T., Lai, X., Martin, L.D., McDonald, H.G., Mol, D., Meldgaard, M., Munch, K., Stephan, E., Sablin, M., Sommer, R.S., Sipko, T., Scott, E., Suchard, M.A., Tikhonov, A., Willerslev, R., Wayne, R.K., Cooper, A., Hofreiter, M., Sher, A., Shapiro, B., Rahbek, C., Willerslev, E., 2011. Species-specific responses of Late Quaternary megafauna to climate and humans. Nature 479, 359–364. http://dx.doi.org/10.1038/nature10574.
Ludwig, A., Pruvost, M., Reissmann, M., Benecke, N., Brockmann, G.A., Castanos, P., Cieslak, M., Lippold, S., Llorente, L., Malaspinas, A.S., Slatkin, M., Hofreiter, M., 2009. Coat color variation at the beginning of horse domestication. Science 324, 485. http://dx.doi.org/10.1126/science.1172750.
Mariat, D., Taourit, S., Guerin, G., 2003. A mutation in the MATP gene causes the cream coat colour in the horse. Genet. Sel. Evol. 35, 119–133. http://dx.doi.org/10.1051/gse:2002039.
Marklund, L., Moller, M.J., Sandberg, K., Andersson, L., 1996. A missense mutation in the gene for melanocyte-stimulating hormone receptor (MC1R) is associated with the chestnut coat color in horses. Mamm. Genome 7, 895–899. http://dx.doi.org/10.1007/s003359900264.
May, E., 1985. Widerristhöhe und Langknochenmaße bei Pferden - ein immer noch aktuelles Problem. Z. Säugetierkunde 50, 368–382.
McGahern, A.M., Edwards, C.J., Bower, M.A., Heffernan, A., Park, S.D., Brophy, P.O., Bradley, D.G., MacHugh, D.E., Hill, E.W., 2006. Mitochondrial DNA sequence diversity in extant Irish horse populations and in ancient horses. Anim. Genet. 37, 498–502. http://dx.doi.org/10.1111/j.1365-2052.2006.01506.x.
Méniel, P., 2007. Les animaux de La Tène. In: Musée Schwab Bienne MSB and Landesmuseum Zürich LZ (eds) La Tène La recherche, les questions, les réponses. Verlag Museum Schwab, Biel, pp. 61–65.
Méniel, P., 2014. Les restes animaux du site du Mormont. Eclépens et La Sarraz, canton de Vaud, vers 100 avant J.-C. Cahiers d'Archéologie Romande, Lausanne.
Möller, L. (Ed.), 2013. Der gallische Krieg/Gaius Iulius Ceasar. Marixverlag, Wiesbaden.
Montane, L., Bourdelle, E., Bressou, C., 1913. Anatomie régionale des animaux domestiques, I: Cheval. Baillière, Paris.
Müller-Lhotska, U.A., 1984. Das Pferd in der Schweiz. Von der Prähistorie bis zum ausgehenden Mittelalter (PhD thesis History department) University of Zürich, Zürich.
Nuviala, P., Nowell, G., Ughetto, J., Balasse, M., 2014. L'origine des chevaux du site du Mormont (canton de Vaud, Suisse): L'apport des isotopes du carbone, de l'oxygène et du strontium. In: Barral, P., Guillaumet, M.-J., Roulière-Lambert, M.-J., Saracino, M., Vitali, D. (Eds.), Les Celtes et le nord de l'Italie Premier et second âges du fer. Société archéologique de l'Est, Vérone, pp. 603–610.
Oder, E., Hoppe, K. (Eds.), 1924–1927. Corpus hippiatricorum graecorum. B. G. Teubner, Leipzig.
Orlando, L., Ginolhac, A., Zhang, G., Froese, D., Albrechtsen, A., Stiller, M., Schubert, M., Cappellini, E., Petersen, B., Moltke, I., Johnson, P.L., Fumagalli, M., Vilstrup, J.T., Raghavan, M., Korneliussen, T., Malaspinas, A.S., Vogt, J., Szklarczyk, D., Kelstrup, C.D., Vinther, J., Dolocan, A., Stenderup, J., Velazquez, A.M., Cahill, J., Rasmussen, M., Wang, X., Min, J., Zazula, G.D., Seguin-Orlando, A., Mortensen, C., Magnussen, K., Thompson, J.F., Weinstock, J., Gregersen, K., Roed, K.H., Eisenmann, V., Rubin, C.J., Miller, D.C., Antczak, D.F., Bertelsen, M.F., Brunak, S., Al-Rasheid, K.A., Ryder, O., Andersson, L., Mundy, J., Krogh, A., Gilbert, M.T., Kjaer, K., Sicheritz-Ponten, T., Jensen, L.J., Olsen, J.V., Hofreiter, M., Nielsen, R., Shapiro, B., Wang, J., Willerslev, E., 2013. Recalibrating *Equus* evolution using the genome sequence of an early Middle Pleistocene horse. Nature 499, 74–78. http://dx.doi.org/10.1038/nature12323.
Paulus, S., Uerpmann, H.P., 2007. Physikalisch-chemische Untersuchungen an Equidenzähnen aus Kalkriese zur Bestimmung von Knochenerhaltung und Jahreszeit der Schlacht. In: Wilbers-Rost, S., Uerpmann, H.P., Uerpmann, M., Grosskopf, B., Tolksdorf-Lienemann, E. (Eds.), Kalkriese 3: Interdisziplinäre Untersuchungen auf dem Oberesch in Kalkriese, Archäologische Befunde und naturwissenschaftliche Begleituntersuchungen. Verlag Philipp von Zabern, Mainz, pp. 145–156.
Peters, J., 1998. Römische Tierhaltung und Tierzucht. Marie Leidorf GmbH, Rahden/Westf.
Pichler SL, Lassau G, Alt KW, Röder B and Schibler J (eds) (in preparation). Über die Toten zu den Lebenden. Interdisziplinäre Synthese, Basel: ABBS.
Priskin, K., Szabo, K., Tomory, G., Bogacsi-Szabo, E., Csanyi, B., Eordogh, R., Downes, C.S., Rasko, I., 2010. Mitochondrial sequence variation in ancient horses from the Carpathian Basin and possible modern relatives. Genetica 138, 211–218. http://dx.doi.org/10.1007/s10709-009-9411-x.
Pruvost, M., Schwarz, R., Correia, V.B., Champlot, S., Braguier, S., Morel, N., Fernandez-Jalvo, Y., Grange, T., Geigl, E.M., 2007. Freshly excavated fossil bones are best for amplification of ancient DNA. PNAS 104, 739–744. http://dx.doi.org/10.1073/pnas.0610257104.
Rapin, A., Méniel, P., Brunaux, J.-L., 1980. Un sanctuaire gaulois à Gournay-sur-Aronde (Oise) Gallia pp. 1–25.
Reissmann, M., 2009. Die Farben der Pferde. Genetik, Klassifizierung, Charakteristik. Cadmos, Brunsbek.
Reissmann, M., Bierwolf, J., Brockmann, G.A., 2007. Two SNPs in the SILV gene are associated with silver coat colour in ponies. Anim. Genet. 38, 1–6. http://dx.doi.org/10.1111/j.1365-2052.2006.01553.x.
Rieder, S., 2009. Molecular tests for coat colour in horses. J. Anim. Breed. Genet. 126, 415–424. http://dx.doi.org/10.1111/j.1439-0388.2009.00832.x.
Rieder, S., Taourit, S., Mariat, D., Langlois, B., Guerin, G., 2001. Mutations in the agouti (*ASIP*), the extension (*MC1R*), and the brown (*TYRP1*) loci and their association to coat color phenotypes in horses (*Equus caballus*). Mamm. Genome 12, 450–455. http://dx.doi.org/10.1007/s003350020017.
Rosengren Pielberg, G., Golovko, A., Sundstrom, E., Curik, I., Lennartsson, J., Seltenhammer, M.H., Druml, T., Binns, M., Fitzsimmons, C., Lindgren, G., Sandberg, K., Baumung, R., Vetterlein, M., Stromberg, S., Grabherr, M., Wade, C., Lindblad-Toh, K., Ponten, F., Heldin, C.H., Solkner, J., Andersson, L., 2008. A *cis*-acting regulatory mutation causes premature hair graying and susceptibility to melanoma in the horse. Nat. Genet. 40, 1004–1009. http://dx.doi.org/10.1038/ng.185.
Santschi, E.M., Purdy, A.K., Valberg, S.J., Vrotsos, P.D., Kaese, H., Mickelson, J.R., 1998. Endothelin receptor B polymorphism associated with lethal white foal syndrome in horses. Mamm. Genome 9, 306–309.
Schibler, J., Stopp, B., Studer, J., 1999. Haustierhaltung und Jagd. In: Müller, F., Kaenel, G., Lüscher, G. (Eds.), SPM IV: Eisenzeit. Schweizerische Gesellschaft für Ur- und Frühgeschichte, Basel, pp. 116–136.
Schibler, J., Studer, J., 1998. Haustierhaltung und Jagd während der Bronzezeit der Schweiz. In: Hochuli, S., Niffeler, U., Rychener, J. (Eds.), SPM III: Bronzezeit. Gesellschaft für Ur- und Frühgeschichte der Schweiz, Basel, pp. 171–192.
Schubert, M., Jonsson, H., Chang, D., Der Sarkissian, C., Ermini, L., Ginolhac, A., Albrechtsen, A., Dupanloup, I., Foucal, A., Petersen, B., Fumagalli, M., Raghavan, M., Seguin-Orlando, A., Korneliussen, T.S., Velazquez, A.M., Stenderup, J., Hoover, C.A., Rubin, C.J., Alfarhan, A.H., Alquraishi, S.A., Al-Rasheid, K.A., MacHugh, D.E., Kalbfleisch, T., MacLeod, J.N., Rubin, E.M., Sicheritz-Ponten, T., Andersson, L., Hofreiter, M., Marques-Bonet, T., Gilbert, M.T., Nielsen, R., Excoffier, L., Willerslev, E., Shapiro, B., Orlando, L., 2014. Prehistoric genomes reveal the genetic foundation and cost of horse domestication. PNAS 111, E5661–E5669. http://dx.doi.org/10.1073/pnas.1416991111.
Senese, C., Penedo, M.C.T., Shiue, Y.L., Bowling, A.T., Millon, L.V., 1999. A HaeIII PCR-RFLP in the ZFY/ZFX genes of horses. Anim. Genet. 30, 390–391. http://dx.doi.org/10.1046/j.1365-2052.1999.00526-10.x.
Shapiro, B., Hofreiter, M. (Eds.), 2012. Ancient DNA - Methods and Protocols. Springer, New York.
Sidnell, P., 2006. Warhorse. Cavalry in Ancient Warfare. Continuum Books, London, New York.

Slovak, N.M., Paytan, A., 2011. Applications of Sr isotopes in archaeology. In: Baskaran, M. (Ed.), Handbook of Environmental Isotope Geochemistry. Springer, Berlin, Heidelberg, pp. 743–768.

Svensson, E.M., Telldahl, Y., Sjoling, E., Sundkvist, A., Hulth, H., Sjovold, T., Gotherstrom, A., 2012. Coat colour and sex identification in horses from Iron Age Sweden. Ann. Anat. 194, 82–87. http://dx.doi.org/10.1016/j.aanat.2011.11.001.

Trut, L., Oskina, I., Kharlamova, A., 2009. Animal evolution during domestication: the domesticated fox as a model. BioEssays 31, 349–360. http://dx.doi.org/10.1002/bies.200800070.

Vila, C., Leonard, J.A., Gotherstrom, A., Marklund, S., Sandberg, K., Liden, K., Wayne, R.K., Ellegren, H., 2001. Widespread origins of domestic horse lineages. Science 291, 474–477. http://dx.doi.org/10.1126/science.291.5503.474.

von den Driesch, A., 1976. A Guide to the Measurement of Animal Bones From Archaeological Sites. Harvard University Press, Cambridge, Mass.

Wallner, B., Vogl, C., Shukla, P., Burgstaller, J.P., Druml, T., Brem, G., 2013. Identification of genetic variation on the horse y chromosome and the tracing of male founder lineages in modern breeds. PLoS One 8, e60015. http://dx.doi.org/10.1371/journal.pone.0060015.

Wappmann, U., 1985. Buch 16 und 17 der Geoponica. Übersetzung und Besprechung (PhD thesis Veterinary Medicine. München).

Weber, C., 2005. Molekulargenetische Typisierung einer skythenzeitlichen Pferdepopulation (Master thesis Paleogenomics Mainz: Johannes Gutenberg-Universität).

Weinstock, J., Willerslev, E., Sher, A., Tong, W., Ho, S.Y., Rubenstein, D., Storer, J., Burns, J., Martin, L., Bravi, C., Prieto, A., Froese, D., Scott, E., Xulong, L., Cooper, A., 2005. Evolution, systematics, and phylogeography of Pleistocene horses in the new world: a molecular perspective. PLoS Biol. 3, e241. http://dx.doi.org/10.1371/journal.pbio.0030241.

Xu, X., Arnason, U., 1994. The complete mitochondrial DNA sequence of the horse, *Equus caballus*: extensive heteroplasmy of the control region. Gene 148, 357–362.

Electronic supplementary material

Table S1: Modern horse information including breed name and origin, number of samples, group assignment and references or GenBank ID in case of unpublished papers. *three specimens with missing data were excluded (HQ153733; HQ153734; HQ153738).

Table S2: Haplogroup definition following Cieslak *et al.* (2010).

Table S3: Parameters for weighting of nucleotide positions for Median Joining Network analysis based on 14 ancient and 355 modern mitochondrial d-loop sequences.

Table S4: Summary statistics of Iron Age horses and modern breeds. Given are number of samples (n), number of loci without missing data (loci), number of segregating sites (S), haplotype diversity and nucleotide diversity (π).

Table S5: Fixation indices (F_{ST}) and *p*-values for Iron Age horses and modern breeds based on pairwise differences.

Table S6: Fixation indices (F_{ST}) and *p*-values for Iron Age horses and modern breeds grouped according to size and origin based on pairwise differences.

Figure S1: Results of PCR amplification with primer systems Both_f/Dup_r (left panel) and Both_f/Both_r (right panel) for Freiberger (FM) and Camargue (CA) horses. Pictures represent Freiberger stallion Cardinal (fm-ch.ch) and Camargue stallion Ugo du Pous (maspousaraque.com).

Figure S2: PCA plot based on relative mtDNA haplotype frequencies. The two dimensions display 48 % of the total variance. The contribution (loading) of each haplotype is presented by bar charts for component 1 and 2.

Table S1: Modern horse information including breed name and origin, number of samples, group assignment and references or GenBank ID in case of unpublished papers. *three specimens with missing data were excluded (HQ153733; HQ153734; HQ153738).

Breed	Origin	Number of samples	Type	References or GenBank ID
Bardigiano	Italy	18	Horse	Bigi *et al.* (2014)
Black Forest	Germany	54	Horse	KC147073-KC147126
Comtois	Eastern France	25	Horse	KC147179-KC147203
Connemara	Ireland	22	Pony	Hill *et al.* (2002), Prystupa *et al.* (2012)
Dale	Great Britain	12	Pony	Prystupa *et al.* (2012)
Dartmoor	Great Britain	12	Pony	Prystupa *et al.* (2012)
Eriskay	Great Britain	12	Pony	Prystupa *et al.* (2012)
Exmoor	Great Britain	23	Pony	Jansen *et al.* (2002), Prystupa *et al.* (2012)
Fjord	Norway	22	Pony	Jansen *et al.* (2002), Prystupa *et al.* (2012)
Freiberger	Switzerland	18	Horse	KC147043; KC147048; KC147053; KC147058-KC147073
Garrano	Spain	10	Pony	Royo *et al.* (2005)
Giara	Italy	25	Pony	Morelli *et al.* (2014)
Haflinger	Italy	28	Pony	Bigi *et al.* (2014)
Highland	Great Britain	11	Pony	Prystupa *et al.* (2012)
Hucul	Carpathians	10	Pony	Priskin *et al.* (2010)
Icelandic	Iceland	modern 22 museum 35*	Pony	Campana *et al.* (2012)
Italian Heavy Draught	Italy	27	Horse	Bigi *et al.* (2014)
Kerry Bog	Ireland	49	Pony	McGahern *et al.* (2006b), Prystupa *et al.* (2012)
Maremmano	Italy	25	Horse	Bigi *et al.* (2014)
Marismeño	Spain	12	Horse	Royo *et al.* (2005)
Medjimurje	Croatia	55	Horse	JQ520291-JQ520345
Murgese	Italy	23	Horse	Bigi *et al.* (2014)

Pottoka	Spain	13	Pony	Royo *et al.* (2005)
Pura Raza Española	Spain	41	Horse	Jansen *et al.* (2002), Lopes *et al.* (2005), Royo *et al.* (2005)
Sarcidano	Italy	18	Horse	Morelli *et al.* (2014)
Shetland	Great Britain	75	Pony	Hill *et al.* (2002), Bower *et al.* (2011)
Ventasso	Italy	36	Horse	Bigi *et al.* (2014)
Vyatskay	Volga region, Russia	18	Pony	McGahern *et al.* (2006a)
Welsh	Great Britain	10	Pony	Prystupa *et al.* (2012)

References

Bigi D, Perrotta G and Zambonelli P (2014) Genetic analysis of seven Italian horse breeds based on mitochondrial DNA D-loop variation. *Anim Genet* 45: 593-595. doi: 10.1111/age.12156

Bower MA, Campana MG, Whitten M, Edwards CJ, Jones H, Barrett E, Cassidy R, Nisbet RE, Hill EW, Howe CJ and Binns M (2011) The cosmopolitan maternal heritage of the Thoroughbred racehorse breed shows a significant contribution from British and Irish native mares. *Biol Lett* 7: 316-320. doi: 10.1098/rsbl.2010.0800

Campana MG, Stock F, Barrett E, Benecke N, Barker GW, Seetah K and Bower MA (2012) Genetic stability in the Icelandic horse breed. *Anim Genet* 43: 447-449. doi: 10.1111/j.1365-2052.2011.02266.x

Hill EW, Bradley DG, Al-Barody M, Ertugrul O, Splan RK, Zakharov I and Cunningham EP (2002) History and integrity of thoroughbred dam lines revealed in equine mtDNA variation. *Anim Genet* 33: 287-294.

Jansen T, Forster P, Levine MA, Oelke H, Hurles M, Renfrew C, Weber J and Olek K (2002) Mitochondrial DNA and the origins of the domestic horse. *PNAS* 99: 10905-10910. doi: 10.1073/pnas.152330099

Lopes MS, Mendonça D, Cymbron T, Valera M, Da Costa-Ferreira J and Da Câmara Machado A (2005) The Lusitano horse maternal lineage based on mitochondrial D-loop sequence variation. *Anim Genet* 36: 196-202. doi: 10.1111/j.1365-2052.2005.01279.x

McGahern A, Bower MAM, Edwards CJ, Brophy PO, Sulimova G, Zakharov I, Vizuete-Forster M, Levine M, Li S, MacHugh DE and Hill EW (2006a) Evidence for biogeographic pattering of mitochondrial DNA sequences in Eastern horse populations. *Anim Genet* 37: 494-497. doi: 10.1111/j.1365-2052.2006.01495.x

McGahern AM, Edwards CJ, Bower MA, Heffernan A, Park SD, Brophy PO, Bradley DG, MacHugh DE and Hill EW (2006b) Mitochondrial DNA sequence diversity in extant

Irish horse populations and in ancient horses. *Anim Genet* 37: 498-502. doi: 10.1111/j.1365-2052.2006.01506.x

Morelli L, Useli A, Sanna D, Barbato M, Contu D, Pala M, Cancedda M and Francalacci P (2014) Mitochondrial DNA lineages of Italian Giara and Sarcidano horses. *Gen Mol Res* 13: 8241-8257. doi: 10.4238/2014.October.20.1

Priskin K, Szabo K, Tomory G, Bogacsi-Szabo E, Csanyi B, Eordogh R, Downes CS and Rasko I (2010) Mitochondrial sequence variation in ancient horses from the Carpathian Basin and possible modern relatives. *Genetica* 138: 211-218. doi: 10.1007/s10709-009-9411-x

Prystupa JM, Hind P, Cothran EG and Plante Y (2012) Maternal lineages in native Canadian equine populations and their relationship to the Nordic and Mountain and Moorland pony breeds. *J Hered* 103: 380-390. doi: 10.1093/jhered/ess003

Royo LJ, Alvarez I, Beja-Pereira A, Molina A, Fernandez I, Jordana J, Gomez E, Gutierrez JP and Goyache F (2005) The origins of Iberian horses assessed via mitochondrial DNA. *J Hered* 96: 663-669. doi: 10.1093/jhered/esi116

Table S2: Haplogroup definition following Cieslak *et al.* (2010).

Haplogroup	Defining sequence pattern, reference NC_001640 (Xu *et al.*, 1994); 16'xxx
A	495C; 602T; 720A
B	495C; 602T; 617C; 720A
D	Reference or 495C
F	495C; 601C; 602T; 720A
I	495C; 538G; 602T; 720A
G	495C; 521A; 602T; 720A; 737C
H	deletion at 532
K	495C; 602T; 703C; 720A; 740G
X2	494C; 495C; 496G; 534T; 720A
X3	495C; 542T; 602T; 635T; 666A; 703C; 720A
X4	495C; 526C; 540G
X7	495C; 602T; 615G; 616G; 703C; 720A

Table S3: Parameters for weighting of nucleotide positions for Median Joining Network analysis based on 14 ancient and 355 modern mitochondrial d-loop sequences.

	1	2	3	12	14	28	29	33	34	36	39	40	41	43	45	47	48	49	50	53	76	81	82	83	84	91
Positions in the reference sequence NC_001640	15494	15495	15496	15505	15507	15521	15522	15526	15527	15529	15532	15533	15534	15536	15538	15540	15541	15542	15543	15546	15569	15574	15575	15576	15577	15584
Nucleotide sequence of reference NC_001640	t	c	a	t	t	g	a	t	a	c	c	a	c	t	a	a	c	c	a	c	t	g	g	c	c	c
Nucleotide sequence of majority	t	t	a	t	t	g	a	t	a	c	c	a	c	t	a	a	c	c	a	c	t	g	g	c	c	c
Number of changes to A						5																2				
Number of changes to T		7			1	1				1			78				2	42		1					1	1
Number of changes to C	78							11	1					1					1				1			
Number of changes to G		1	78	2			2					2			17	10					1			1		
Number of deletions											5	1														
Number of insertions																										
Number of transitions	78	7	78		1	5	2	11		1		2	78	1	17	10	2	42		1		2			1	1
Number of transversions		1		2		1			1										1		1		1	1		
Number of differences to majority (n=301)	78	8	78	2	1	6	2	11	1	1	x	x	78	1	17	10	2	42	1	1	1	2	1	1	1	1
Weight (positions without polymorphisms are not included in Network analysis	11	46	11	49	50	47	49	45	50	50	99	99	11	50	42	45	49	29	50	50	50	49	50	50	50	50
	92	96	103	104	105	106	107	108	109	110	111	120	122	123	124	125	126	130	133	134	138	139	140	141	142	146
Positions in the reference sequence NC_001640	15585	15589	15596	15597	15598	15599	15600	15601	15602	15603	15604	15613	15615	15616	15617	15618	15619	15623	15626	15627	15631	15632	15633	15634	15635	15639
Nucleotide sequence of reference NC_001640	g	t	a	a	t	t	g	t	t	t	g	a	a	a	t	a	a	t	a	c	a	t	a	t	c	t
Nucleotide sequence of majority	g	t	a	a	t	t	g	t	c	t	g	a	a	a	t	a	a	t	a	c	a	t	a	t	c	t
Number of changes to A	140						3				58									1					1	1
Number of changes to T																							1		36	
Number of changes to C	1	1			5			18	68	79					23	1	1					1		1		
Number of changes to G		1	4	55		1		1		1		2	10	7				2	1		1					
Number of deletions																										
Number of insertions																										
Number of transitions	140	1	4	55	5		3	18	68	79	58	2	10	7	23				1		1	1		1	36	
Number of transversions	1	1				1		1		1						1	1	2		1			1		1	1
Number of differences to majority (n=301)	141	2	4	55	5	1	3	19	68	80	58	2	10	7	23	1	1	2	1	1	1	1	1	1	37	1
Weight (positions without polymorphisms are not included in Network analysis	0	49	48	23	48	50	49	41	16	10	21	49	45	47	39	50	50	49	50	50	50	50	50	50	32	50
	151	156	157	158	164	166	172	173	174	176b	182	184	185	189	190	193	197	198	199	201	207	213	218	221		
Positions in the reference sequence NC_001640	15644	15649	15650	15651	15657	15659	15665	15666	15667		15701	15703	15704	15708	15709	15712	15716	15717	15718	15720	15726	15732	15737	15740		
Nucleotide sequence of reference NC_001640	t	a	a	g	t	t	t	g	a	_	a	t	c	a	c	a	c	a	c	a	g	c	t	a		
Nucleotide sequence of majority	t	a	a	g	t	t	t	g	a	_	a	t	c	a	c	a	c	a	c	g	g	c	t	a		
Number of changes to A	2			1				67					1		1		1				15				299	
Number of changes to T									1						15				11					2	201	
Number of changes to C					1	25			1			94											5	1	419	
Number of changes to G		84	96				1		13		1			2	2	1		1		15		1		26	443	
Number of deletions																									6	
Number of insertions										3															3	
Number of transitions		84	96	1	1	25		67	13		1	94		2	15	1		1	11	15	15		5	26	1326	
Number of transversions	2						1		2				1		3		1					1		3	36	
Number of differences to majority (n=301)	2	84	96	1	1	25	1	67	15	x	1	94	1	2	18	1	1	1	11	15	15	1	5	29	1360	
Weight (positions without polymorphisms are not included in Network analysis	49	8	2	50	50	38	50	17	45	99	50	3	50	49	41	50	50	50	45	43	43	50	48	36		

Table S4: Summary statistics of Iron Age horses and modern breeds. Given are number of samples (n), number of loci without missing data (loci), number of segregating sites (S), haplotype diversity and nucleotide diversity (π).

Name	n	Loci	S	Haplotype diversity	π
Iron Age	14	211	20	0.9231±0.0604	0.029634±0.016805
Bardigiano	18	247	21	0.9935±0.0210	0.027705±0.015300
Black Forest	54	247	23	0.8567±0.0289	0.024144±0.012976
Comtois	25	247	17	0.9400±0.0235	0.021161±0.011800
Connemara	22	248	21	0.9524±0.0291	0.024927±0.013741
Dale	12	247	14	0.5758±0.1634	0.015765±0.009574
Dartmoor	12	247	10	0.4394±0.1581	0.014722±0.009028
Eriskay	12	247	12	0.5909±0.1079	0.017667±0.010570
Exmoor	23	247	18	0.7352±0.0775	0.021507±0.012017
Fjord	22	247	22	0.8874±0.0430	0.026184±0.014372
Freiberger	18	247	22	0.9542±0.0394	0.025271±0.014075
Garrano	10	247	16	0.9778±0.0540	0.025461±0.014929
Giara	25	248	32	0.9433±0.0366	0.020591±0.011512
Haflinger	28	247	27	0.9656±0.0244	0.027194±0.014717
Highland	11	247	15	0.8364±0.0887	0.027236±0.015698
Hucul	10	247	10	0.7111±0.1175	0.016734±0.010283
Iceland all	57	247	19	0.8778±0.0267	0.021892±0.011874
Iceland modern	22	247	16	0.8961±0.0408	0.022977±0.012776
Iceland museum	35	247	19	0.8706±0.0354	0.021338±0.011742
Italian HD	27	247	31	0.9829±0.0154	0.025745±0.014024
Kerry Bog	49	247	26	0.9209±0.0173	0.021289±0.011618
Maremmano	25	247	26	0.9900±0.0142	0.028448±0.015405
Marismeno	12	247	14	0.8485±0.0744	0.024107±0.013929
Medjimurje	55	247	28	0.9333±0.0152	0.027997±0.014832
Murgese	23	247	21	0.9644±0.0224	0.023651±0.013083
Pottoka	13	247	15	1.0000±0.0302	0.017440±0.010374
Pura Raza ESP	41	247	24	0.9341±0.0188	0.024686±0.013320
Sarcidano	18	248	36	0.9608±0.0335	0.032838±0.017875
Shetland	75	247	26	0.8555±0.0232	0.024624±0.013137
Ventasso	36	247	23	0.8794±0.0463	0.025101±0.013571
Vyatskaya	18	247	17	0.9281±0.0373	0.021116±0.011982
Welsh	10	247	16	1.0000±0.0447	0.023122±0.013686

Table S5: Fixation indices (F_{ST}) and p-values for Iron Age horses and modern breeds based on pairwise differences.

	Iron Age	Bardigiano	Black Forest	Comtois	Connemara	Dale	Dartmoor	Eriskay	Exmoor	Fjord	Freiberger	Garrano	Giara	Haflinger	Highland	Hucul	Iceland all	Iceland modern	Iceland museum	Italian Heavy Draught	Kerry Bog	Maremmano	Marismeno	Medjimurje	Murgese	Pottoka	Pura Raza Espanola	Sarcidano	Shetland	Ventasso	Vyatskaya	Welsh
Iron Age	0																															
Bardigiano	0.01746	0																														
Black Forest	0.04607	0.02341	0																													
Comtois	0.09068	0.05116	0.00331	0																												
Connemara	0.03084	0	0.00044	0.00451	0																											
Dale	0.191	0.21946	0.20377	0.21541	0.20717	0																										
Dartmoor	0.23365	0.14966	0.22654	0.2591	0.12758	0.45012	0																									
Eriskay	0.18852	0.20454	0.21202	0.24086	0.20255	0.04414	0.41582	0																								
Exmoor	0.07397	0.16488	0.22231	0.22989	0.1917	0.24375	0.31936	0.27052	0																							
Fjord	0.01802	0.00577	0.02788	0.02978	0.02883	0.16299	0.23099	0.18814	0.14304	0																						
Freiberger	0	0.00574	0.02998	0.04157	0.00576	0.174	0.17978	0.159	0.0828	0.0145	0																					
Garrano	0	0	0	0.03618	0.00501	0.21515	0.25393	0.19587	0.14452	0	0	0																				
Giara	0.25381	0.35059	0.3887	0.47425	0.41876	0.49914	0.56404	0.5055	0.35228	0.36166	0.35835	0.31828	0																			
Haflinger	0.03042	0.01885	0	0	0.00907	0.19435	0.229	0.2257	0.20263	0.01653	0.03797	0.0145	0.37184	0																		
Highland	0.15632	0.03102	0.05478	0.05525	0.06847	0.25547	0.29015	0.23229	0.32649	0.07411	0.11104	0.05143	0.48021	0.0402	0																	
Hucul	0.12371	0.16291	0.23035	0.28351	0.23899	0.3655	0.45476	0.3422	0.19327	0.1773	0.16879	0.12858	0.33455	0.2428	0.31123	0																
Iceland all	0.06831	0.06253	0.11733	0.09486	0.10213	0.13515	0.27427	0.16594	0.1502	0.05093	0.06041	0.03745	0.41545	0.11055	0.12137	0.15539	0															
Iceland modern	0.08696	0.04503	0.10729	0.08441	0.0971	0.15845	0.28998	0.19311	0.189	0.04343	0.08195	0.03282	0.42128	0.08883	0.0716	0.16795	0	0														
Iceland museum	0.0476	0.06524	0.11811	0.10045	0.09853	0.12729	0.27866	0.1527	0.12151	0.0489	0.03899	0.03476	0.41467	0.1144	0.14315	0.15283	0	0	0													
Italian Heavy Draught	0	0	0.04762	0.0833	0.02939	0.19052	0.2054	0.18483	0.1036	0.00544	0	0	0.3063	0.05542	0.13509	0.12445	0.06704	0.07868	0.05197	0												
Kerry Bog	0.07863	0.11232	0.15603	0.15157	0.11766	0.06972	0.22754	0.10634	0.08595	0.08317	0.0438	0.10158	0.42892	0.15444	0.22149	0.22255	0.07129	0.10642	0.04571	0.08044	0											
Maremmano	0	0.02361	0.08061	0.12516	0.08212	0.18883	0.23972	0.20118	0.08927	0.03632	0.02672	0	0.18581	0.06957	0.14884	0.07621	0.08966	0.08668	0.08058	0.01076	0.10928	0										
Marismeno	0.00553	0.01757	0.00215	0.06442	0.01997	0.2629	0.27311	0.25261	0.21097	0	0.01773	0	0.34245	0.01841	0.10355	0.2585	0.14927	0.14878	0.14386	0.00251	0.16177	0.03863	0									
Medjimurje	0.01635	0.03098	0.07284	0.06763	0.06539	0.1414	0.22979	0.12321	0.11238	0.01214	0.03111	0	0.32248	0.06011	0.09019	0.12627	0.03838	0.03357	0.03385	0.02838	0.09083	0.03899	0.05613	0								
Murgese	0.04453	0.00603	0.05625	0.04753	0.04468	0.18458	0.25067	0.18145	0.12351	0.02995	0.00824	0	0.38527	0.05298	0.07608	0.12234	0.02115	0.01854	0.01879	0.03049	0.08987	0.04329	0.08551	0.01761	0							
Pottoka	0.24991	0.15558	0.1006	0.11803	0.10335	0.43172	0.33834	0.42396	0.43335	0.17934	0.20469	0.20732	0.57082	0.08188	0.1048	0.50751	0.3041	0.28802	0.3229	0.23014	0.33846	0.26561	0.15284	0.2203	0.2491	0						
Pura Raza Espanola	0.0704	0.04163	0	0.01355	0.02001	0.21919	0.26689	0.24238	0.25021	0.02816	0.06777	0.01751	0.4039	0	0.06203	0.27443	0.14556	0.12818	0.14955	0.06474	0.18741	0.10781	0.00336	0.08797	0.08971	0.08195	0					
Sarcidano	0	0.03502	0.06581	0.07655	0.0482	0.16916	0.25804	0.16898	0.11026	0.03719	0.02163	0.001	0.31716	0.05553	0.12809	0.17019	0.11355	0.11267	0.09656	0.02055	0.11275	0.05645	0.03412	0.05954	0.04959	0.22297	0.06034	0				
Shetland	0.08623	0.05322	0.08795	0.0842	0.07689	0.10863	0.22456	0.08985	0.17145	0.06133	0.02515	0.03917	0.42123	0.09965	0.07551	0.19859	0.05092	0.05974	0.03999	0.07566	0.05221	0.10652	0.10879	0.07058	0.03339	0.23157	0.12483	0.10072	0			
Ventasso	0.16323	0.08541	0.06562	0.04472	0.07412	0.26359	0.29623	0.29307	0.29691	0.08467	0.13848	0.09066	0.44777	0.04285	0.05839	0.25889	0.1592	0.11923	0.17579	0.14893	0.24216	0.15349	0.12862	0.11994	0.088	0.14205	0.06404	0.1498	0.15966	0		
Vyatskaya	0.01716	0	0.017	0.05487	0	0.24097	0.19541	0.2115	0.18537	0.0077	0	0	0.41042	0.04072	0.10147	0.223	0.09783	0.10961	0.08877	0	0.10313	0.05772	0	0.04905	0.03494	0.19217	0.04547	0.04659	0.05274	0.12422		
Welsh	0.10386	0.04711	0.00811	0	0	0.27046	0.24642	0.2978	0.27095	0.04829	0.06996	0.07692	0.49221	0	0.04231	0.37535	0.16896	0.1494	0.17742	0.10343	0.19296	0.14408	0.05114	0.10503	0.10315	0.0062	0	0.07849	0.12483	0.04168	0.06791	0
significance code	< 0.001	< 0.01	< 0.05																													

	Iron Age	Bardigiano	Black Forest	Comtois	Connemara	Dale	Dartmoor	Eriskay	Exmoor	Fjord	Freiberger	Garrano	Giara	Haflinger	Highland	Hucul	Iceland all	Iceland modern	Iceland museum	Italian HD	Kerry Bog	Maremmano	Marismeno	Medjimurje	Murgese	Pottoka	Pura Raza ESP	Sarcidano	Shetland	Ventasso	Vyatskaya	Welsh
Iron Age	*																															
Bardigiano	0.24108± 0.0032	*																														
Black Forest	0.07949± 0.0022	0.13554± 0.0029	*																													
Comtois	0.03493± 0.0014	0.06736± 0.0020	0.30813± 0.0036	*																												
Connemara	0.16016± 0.0029	0.40574± 0.0037	0.35362± 0.0038	0.30032± 0.0041	*																											
Dale	0.00144± 0.0003	0.00044± 0.0002	0.00062± 0.0002	0.00306± 0.0004	0.00125± 0.0003	*																										
Dartmoor	0.00075± 0.0002	0.01568± 0.0010	0.00031± 0.0001	0.00137± 0.0003	0.02787± 0.0013	0.00000± 0.0000	*																									
Eriskay	0.00062± 0.0002	0.00062± 0.0002	0.00037± 0.0001	0.00069± 0.0002	0.00044± 0.0002	0.19403± 0.0035	0.00000± 0.0000	*																								
Exmoor	0.03599± 0.0015	0.00000± 0.0000	0.00000± 0.0000	0.00000± 0.0000	0.00000± 0.0000	0.00000± 0.0000	0.00000± 0.0000	0.00000± 0.0000	*																							
Fjord	0.22646± 0.0031	0.31313± 0.0036	0.09886± 0.0023	0.13141± 0.0026	0.13947± 0.0028	0.00537± 0.0006	0.00237± 0.0004	0.00081± 0.0002	0.00019± 0.0001	*																						
Freiberger	0.62626± 0.0039	0.32294± 0.0033	0.11060± 0.0024	0.10642± 0.0022	0.31244± 0.0038	0.00112± 0.0003	0.00544± 0.0006	0.00194± 0.0004	0.01300± 0.0009	0.23714± 0.0031	*																					
Garrano	0.63519± 0.0038	0.83834± 0.0029	0.39468± 0.0037	0.16416± 0.0029	0.32631± 0.0038	0.00512± 0.0006	0.00487± 0.0006	0.00237± 0.0004	0.00506± 0.0005	0.78404± 0.0033	0.64144± 0.0036	*																				
Giara	0.00025± 0.0001	0.00000± 0.0000	0.00000± 0.0000	0.00000± 0.0000	0.00000± 0.0000	0.00000± 0.0000	0.00000± 0.0000	0.00000± 0.0000	0.00000± 0.0000	0.00000± 0.0000	0.00000± 0.0000	0.00019± 0.0001	*																			
Haflinger	0.14622± 0.0026	0.18378± 0.0033	0.41086± 0.0038	0.35843± 0.0036	0.25077± 0.0035	0.00137± 0.0003	0.00131± 0.0003	0.00025± 0.0001	0.00000± 0.0000	0.19371± 0.0032	0.09642± 0.0024	0.24158± 0.0033	0.00000± 0.0000	*																		
Highland	0.01712± 0.0010	0.19321± 0.0029	0.07392± 0.0021	0.11667± 0.0023	0.07217± 0.0019	0.00612± 0.0006	0.00369± 0.0005	0.00819± 0.0007	0.00000± 0.0000	0.06924± 0.0021	0.02587± 0.0013	0.17565± 0.0028	0.00000± 0.0000	0.13491± 0.0030	*																	
Hucul	0.02293± 0.0012	0.00381± 0.0005	0.00075± 0.0002	0.00056± 0.0002	0.00044± 0.0002	0.00000± 0.0000	0.00000± 0.0000	0.00000± 0.0000	0.00225± 0.0004	0.00269± 0.0004	0.00281± 0.0004	0.03574± 0.0015	0.00000± 0.0000	0.00112± 0.0003	0.00144± 0.0003	*																
Iceland all	0.02737± 0.0014	0.02262± 0.0013	0.00006± 0.0001	0.00225± 0.0004	0.00169± 0.0003	0.00406± 0.0005	0.00000± 0.0000	0.00137± 0.0003	0.00000± 0.0000	0.02650± 0.0012	0.02456± 0.0011	0.14141± 0.0027	0.00000± 0.0000	0.00069± 0.0002	0.01225± 0.0008	0.00325± 0.0005	*															
Iceland modern	0.03024± 0.0013	0.09923± 0.0024	0.00244± 0.0004	0.02612± 0.0012	0.00994± 0.0008	0.00687± 0.0007	0.00012± 0.0001	0.00169± 0.0003	0.00000± 0.0000	0.09105± 0.0023	0.02562± 0.0013	0.18903± 0.0028	0.00000± 0.0000	0.01600± 0.0010	0.10373± 0.0023	0.00550± 0.0007	0.83403± 0.0031	*														
Iceland museum	0.07186± 0.0022	0.02243± 0.0011	0.00019± 0.0001	0.00425± 0.0005	0.00212± 0.0003	0.00431± 0.0005	0.00000± 0.0000	0.00144± 0.0003	0.00000± 0.0000	0.04137± 0.0015	0.07142± 0.0020	0.15828± 0.0026	0.00000± 0.0000	0.00131± 0.0003	0.00844± 0.0007	0.00306± 0.0004	0.95732± 0.0017	0.49091± 0.0046	*													
Italian HD	0.98713± 0.0008	0.45248± 0.0040	0.02793± 0.0014	0.01206± 0.0009	0.10323± 0.0025	0.00050± 0.0002	0.00025± 0.0001	0.00000± 0.0000	0.00037± 0.0002	0.29682± 0.0036	0.57189± 0.0038	0.94014± 0.0018	0.00000± 0.0000	0.02799± 0.0013	0.00725± 0.0006	0.00619± 0.0006	0.00506± 0.0006	0.00944± 0.0008	0.01456± 0.0010	*												
Kerry Bog	0.00637± 0.0007	0.00056± 0.0002	0.00000± 0.0000	0.00000± 0.0000	0.00031± 0.0001	0.02843± 0.0014	0.00006± 0.0001	0.00450± 0.0005	0.00056± 0.0002	0.00237± 0.0004	0.04012± 0.0016	0.00700± 0.0007	0.00000± 0.0000	0.00000± 0.0000	0.00000± 0.0000	0.00006± 0.0001	0.00019± 0.0001	0.00031± 0.0001	0.00781± 0.0007	0.00037± 0.0001	*											
Maremmano	0.58014± 0.0040	0.15891± 0.0030	0.00731± 0.0006	0.00331± 0.0004	0.01306± 0.0009	0.00019± 0.0001	0.00000± 0.0000	0.00025± 0.0001	0.00400± 0.0005	0.08011± 0.0023	0.14229± 0.0029	0.59795± 0.0039	0.00012± 0.0001	0.02193± 0.0012	0.00756± 0.0007	0.04562± 0.0016	0.00144± 0.0003	0.00906± 0.0008	0.00212± 0.0003	0.22708± 0.0035	0.00000± 0.0000	*									
Marismeno	0.35012± 0.0035	0.23739± 0.0035	0.34400± 0.0039	0.07967± 0.0023	0.23239± 0.0033	0.00425± 0.0005	0.00300± 0.0005	0.00087± 0.0003	0.00019± 0.0001	0.39843± 0.0042	0.25920± 0.0032	0.71312± 0.0036	0.00019± 0.0001	0.21896± 0.0035	0.07667± 0.0021	0.00087± 0.0002	0.00219± 0.0004	0.00925± 0.0007	0.00212± 0.0003	0.33650± 0.0036	0.00019± 0.0001	0.13048± 0.0026	*								
Medjimurje	0.19646± 0.0030	0.08223± 0.0022	0.00037± 0.0001	0.00531± 0.0006	0.00569± 0.0005	0.00062± 0.0002	0.00000± 0.0000	0.00206± 0.0004	0.00000± 0.0000	0.20984± 0.0034	0.07761± 0.0019	0.54327± 0.0037	0.00000± 0.0000	0.00675± 0.0007	0.01806± 0.0011	0.00381± 0.0005	0.00844± 0.0007	0.05761± 0.0019	0.02443± 0.0012	0.04968± 0.0016	0.00000± 0.0000	0.02437± 0.0012	0.04718± 0.0017	*							
Murgese	0.08623± 0.0023	0.30482± 0.0032	0.02431± 0.0012	0.06886± 0.0019	0.06855± 0.0020	0.00106± 0.0003	0.00019± 0.0001	0.00037± 0.0001	0.00006± 0.0001	0.11598± 0.0025	0.28626± 0.0030	0.53084± 0.0037	0.00000± 0.0000	0.04818± 0.0015	0.07024± 0.0020	0.01056± 0.0009	0.13404± 0.0025	0.19803± 0.0033	0.15935± 0.0029	0.07767± 0.0022	0.00050± 0.0002	0.05343± 0.0020	0.03937± 0.0016	0.13435± 0.0027	*						
Pottoka	0.00056± 0.0002	0.00575± 0.0006	0.01437± 0.0010	0.01750± 0.0010	0.02093± 0.0012	0.00012± 0.0001	0.00175± 0.0003	0.00000± 0.0000	0.00000± 0.0000	0.00206± 0.0003	0.00131± 0.0003	0.00475± 0.0006	0.00000± 0.0000	0.02968± 0.0015	0.06249± 0.0018	0.00000± 0.0000	0.00000± 0.0000	0.00031± 0.0001	0.00000± 0.0000	0.00012± 0.0001	0.00000± 0.0000	0.00000± 0.0000	0.02493± 0.0013	0.00000± 0.0000	0.00031± 0.0001	*					
Pura Raza ESP	0.04562± 0.0018	0.07774± 0.0023	0.46341± 0.0040	0.19253± 0.0029	0.14547± 0.0029	0.00081± 0.0002	0.00006± 0.0001	0.00006± 0.0001	0.00000± 0.0000	0.11004± 0.0025	0.02987± 0.0013	0.24789± 0.0034	0.00000± 0.0000	0.48197± 0.0038	0.07642± 0.0020	0.00019± 0.0001	0.00000± 0.0000	0.00181± 0.0003	0.00006± 0.0001	0.01712± 0.0010	0.00000± 0.0000	0.00244± 0.0003	0.33806± 0.0039	0.00025± 0.0001	0.00569± 0.0006	0.03106± 0.0013	*				
Sarcidano	0.62876± 0.0033	0.10748± 0.0024	0.02387± 0.0012	0.02456± 0.0012	0.06318± 0.0020	0.00137± 0.0003	0.00019± 0.0001	0.00019± 0.0001	0.00050± 0.0002	0.08780± 0.0023	0.17097± 0.0028	0.36724± 0.0036	0.00000± 0.0000	0.04468± 0.0018	0.01375± 0.0009	0.00169± 0.0004	0.00094± 0.0002	0.00387± 0.0005	0.00237± 0.0004	0.14928± 0.0027	0.00031± 0.0001	0.03012± 0.0014	0.15610± 0.0030	0.00962± 0.0008	0.04599± 0.0015	0.00050± 0.0002	0.03306± 0.0014	*			
Shetland	0.01281± 0.0009	0.03081± 0.0014	0.00019± 0.0001	0.00312± 0.0005	0.00550± 0.0006	0.00987± 0.0007	0.00037± 0.0002	0.01600± 0.0011	0.00000± 0.0000	0.01212± 0.0009	0.12291± 0.0027	0.12466± 0.0030	0.00000± 0.0000	0.00087± 0.0002	0.04237± 0.0016	0.00156± 0.0003	0.00331± 0.0004	0.01812± 0.0010	0.01981± 0.0011	0.00244± 0.0004	0.00219± 0.0004	0.00050± 0.0002	0.00806± 0.0007	0.00000± 0.0000	0.06093± 0.0018	0.00000± 0.0000	0.00000± 0.0000	0.00137± 0.0003	*		
Ventasso	0.00237± 0.0004	0.01637± 0.0009	0.00837± 0.0008	0.05924± 0.0019	0.02343± 0.0011	0.00025± 0.0001	0.00012± 0.0001	0.00006± 0.0001	0.00000± 0.0000	0.01525± 0.0009	0.00325± 0.0004	0.04787± 0.0018	0.00000± 0.0000	0.04199± 0.0017	0.08067± 0.0023	0.00050± 0.0002	0.00000± 0.0000	0.00275± 0.0004	0.00012± 0.0001	0.00044± 0.0002	0.00000± 0.0000	0.00050± 0.0002	0.01275± 0.0009	0.00000± 0.0000	0.01100± 0.0008	0.00400± 0.0005	0.01000± 0.0008	0.00125± 0.0003	0.00000± 0.0000	*		
Vyatskaya	0.25002± 0.0038	0.47672± 0.0040	0.19259± 0.0030	0.07442± 0.0021	0.43561± 0.0041	0.00044± 0.0002	0.00725± 0.0007	0.00075± 0.0002	0.00000± 0.0000	0.28182± 0.0033	0.52534± 0.0039	0.72230± 0.0032	0.00000± 0.0000	0.08798± 0.0023	0.04405± 0.0017	0.00131± 0.0003	0.00312± 0.0005	0.01131± 0.0009	0.00687± 0.0006	0.49316± 0.0040	0.00125± 0.0003	0.04230± 0.0018	0.46660± 0.0038	0.02799± 0.0013	0.10742± 0.0025	0.00275± 0.0004	0.07561± 0.0020	0.06705± 0.0022	0.03243± 0.0015	0.00650± 0.0006	*	
Welsh	0.05455± 0.0019	0.14097± 0.0023	0.30925± 0.0035	0.48122± 0.0042	0.43642± 0.0036	0.00681± 0.0006	0.02375± 0.0011	0.00212± 0.0003	0.00012± 0.0001	0.13091± 0.0025	0.07974± 0.0020	0.09180± 0.0023	0.00000± 0.0000	0.70449± 0.0036	0.20002± 0.0030	0.00037± 0.0001	0.00337± 0.0005	0.01937± 0.0011	0.00350± 0.0004	0.02131± 0.0012	0.00081± 0.0002	0.01131± 0.0009	0.19553± 0.0031	0.01012± 0.0007	0.03699± 0.0015	0.33812± 0.0040	0.49366± 0.0041	0.05668± 0.0019	0.01106± 0.0008	0.14335± 0.0027	0.10486± 0.0027	*

Table S6: Fixation indices (F_{ST}) and p-values for Iron Age horses and modern breeds grouped according to size and origin based on pairwise differences.

	Iron Age	Spanish horses	Spanish ponies	Italian horses	Italian ponies	Croatian horses	Croatian, Carpathian, Russian ponies	Swiss, German, Eastern French horses	Northern European (incl. British Isles) ponies
Iron Age	0								
Spanish horses	0.05686	0							
Spanish ponies	0.09333	0.003	0						
Italian horses	0	0.04877	0.0708	0					
Italian ponies	0.0093	0.10556	0.1402	0.02958	0				
Croatian horses	0.01635	0.08259	0.09564	0.0314	0.09543	0			
Croatian, Carpathian, Russian ponies	0.00545	0.08619	0.10848	0.01486	0.09425	0.03949	0		
Swiss, German, Eastern French horses	0.04144	0.01016	0.01436	0.04032	0.11955	0.06267	0.0526	0	
Northern European (incl. British Isles) ponies	0.03802	0.09354	0.0991	0.05617	0.15018	0.03896	0.04141	0.05004	0
significance code	< 0.001	< 0.01	< 0.05						
	Iron Age	Spanish horses	Spanish ponies	Italian horses	Italian ponies	Croatian horses	Croatian, Carpathian, Russian ponies	Swiss, German, Eastern French horses	Northern European (incl. British Isles) ponies
Iron Age	*								
Spanish horses	0.05755± 0.0018	*							
Spanish ponies	0.02287± 0.0011	0.30169± 0.0035	*						
Italian horses	0.59564± 0.0038	0.00050± 0.0002	0.00250± 0.0004	*					
Italian ponies	0.25258± 0.0035	0.00012± 0.0001	0.00031± 0.0001	0.00625± 0.0007	*				
Croatian horses	0.19840± 0.0030	0.00044± 0.0002	0.00081± 0.0002	0.00237± 0.0004	0.00000± 0.0000	*			
Croatian, Carpathian, Russian ponies	0.35206± 0.0035	0.00431± 0.0005	0.00319± 0.0004	0.09398± 0.0025	0.00169± 0.0003	0.01993± 0.0012	*		
Swiss, German, Eastern French horses	0.07305± 0.0022	0.12860± 0.0024	0.15666± 0.0028	0.00019± 0.0001	0.00000± 0.0000	0.00037± 0.0001	0.01493± 0.0009	*	
Northern European (incl. British Isles) ponies	0.04993± 0.0018	0.00000± 0.0000	0.00006± 0.0001	0.00000± 0.0000	0.00000± 0.0000	0.00062± 0.0002	0.00550± 0.0006	0.00000± 0.0000	*

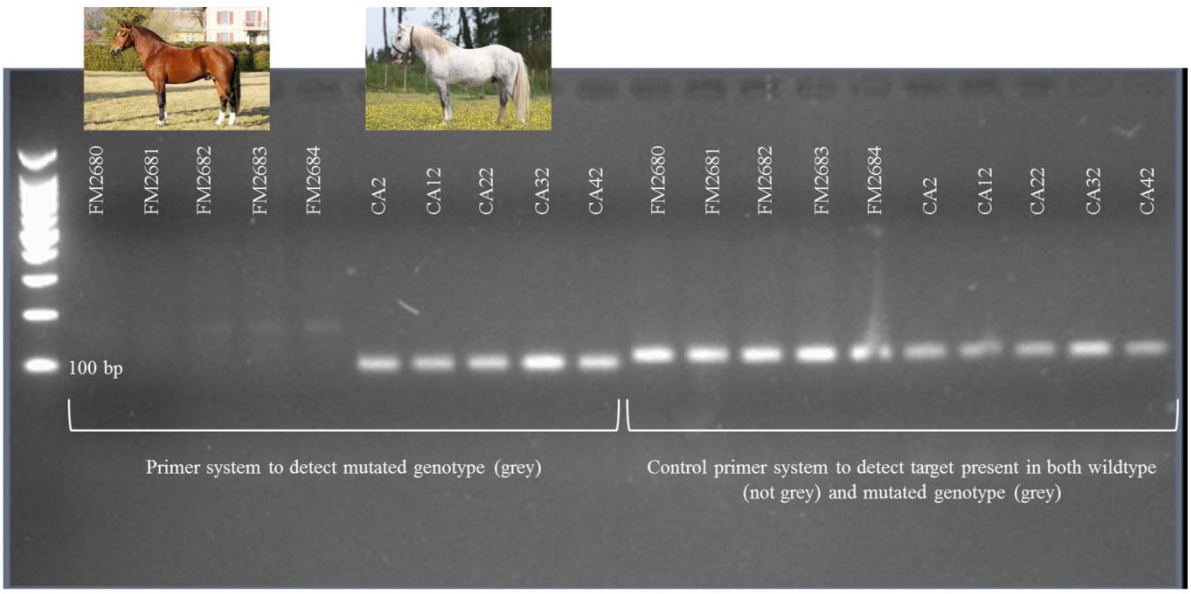
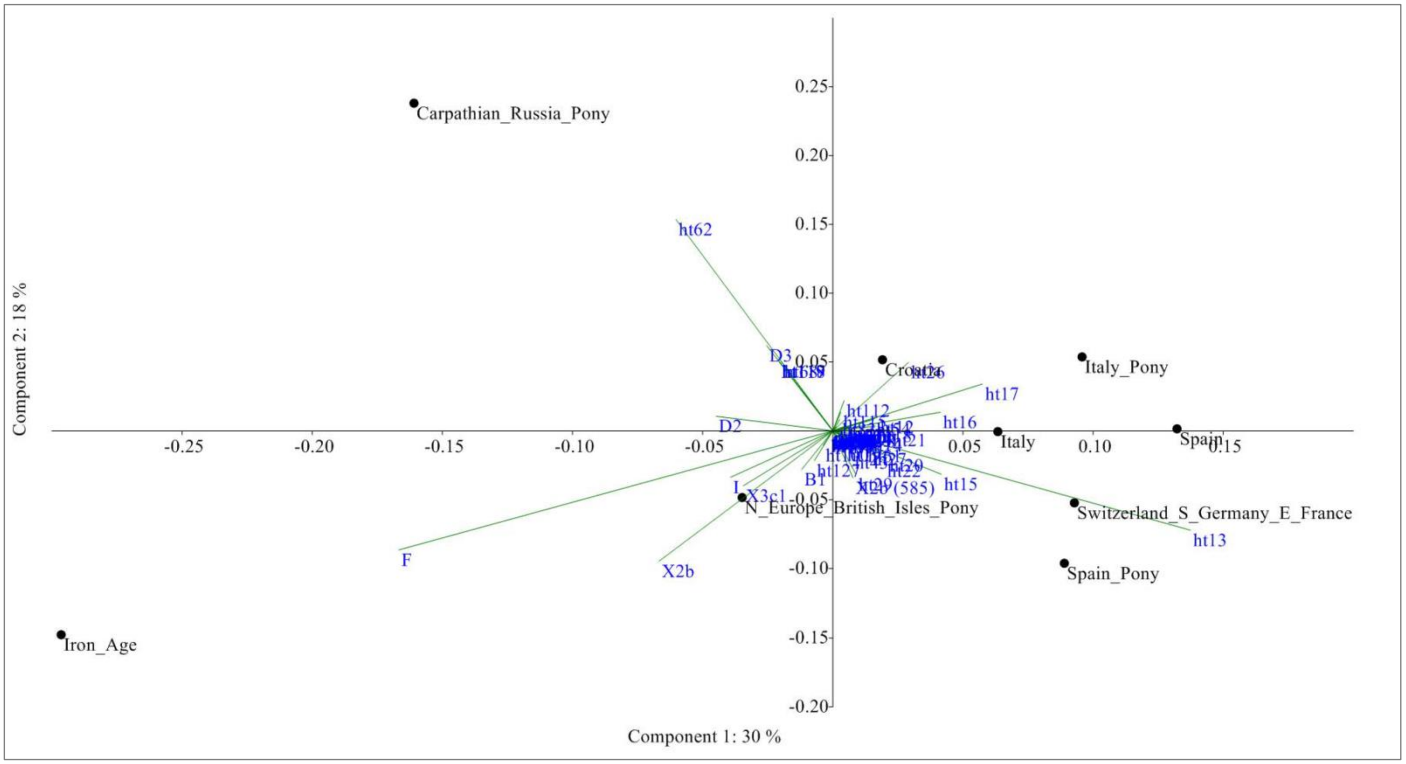
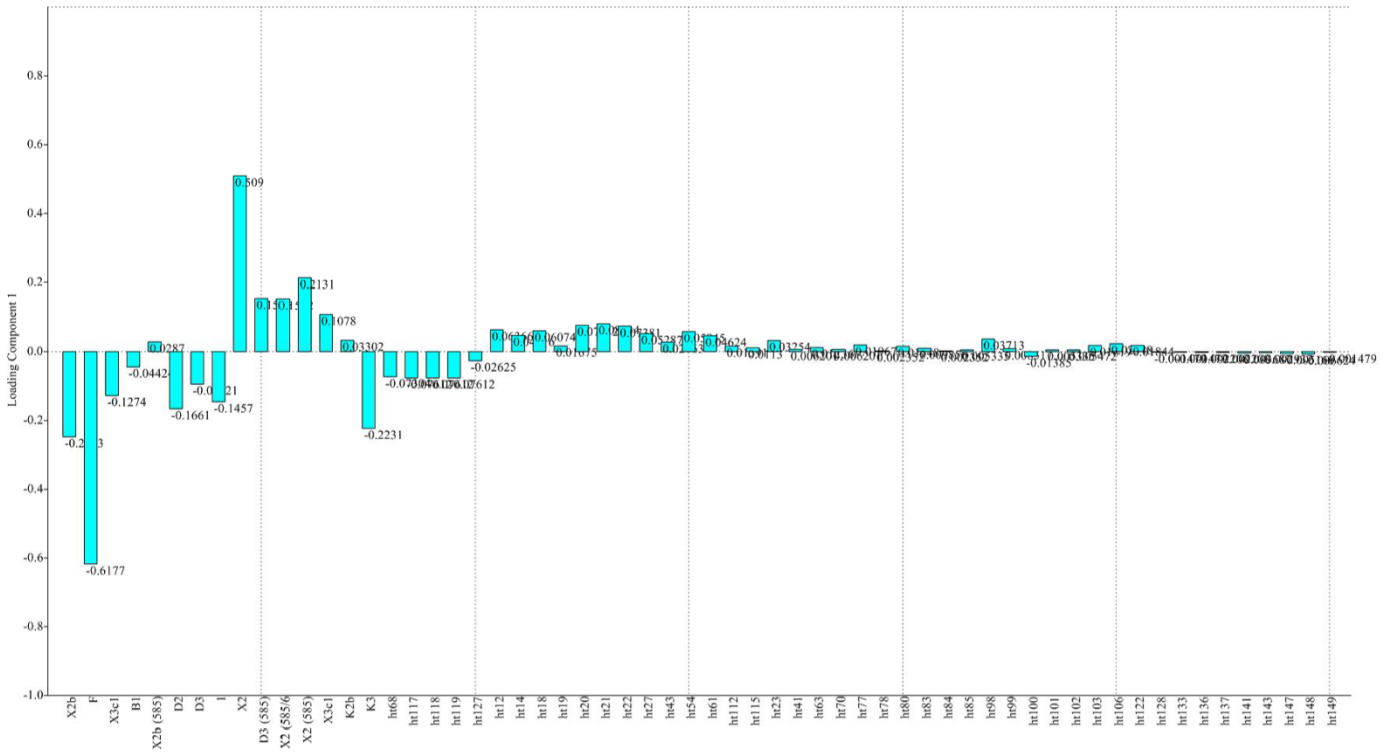


Figure S1: Results of PCR amplification with primer systems Both_f/Dup_r (left panel) and Both_f/Both_r (right panel) for Freiberger (FM) and Camargue (CA) horses. Pictures represent Freiberger stallion Cardinal (fm-ch.ch) and Camargue stallion Ugo du Pous (maspousaraque.com).


Carpathian_Russia_Pony
Italy_Pony
Croatia
Italy
Spain
N_Europe_British_Isles_Pony
Switzerland_S_Germany_E_France
Spain_Pony
Iron_Age
ht62
ht17
ht16
ht15
ht13
D3
D2
B1
I
X3c1
X2b
F
X2 (585)
Component 1: 30 %
Component 2: 18 %


Loading Component 1
X2b
F
X3c1
B1
X2b (585)
D2
D3
I
X2
D3 (585)
X2 (585)
X3c1
K2b
K3

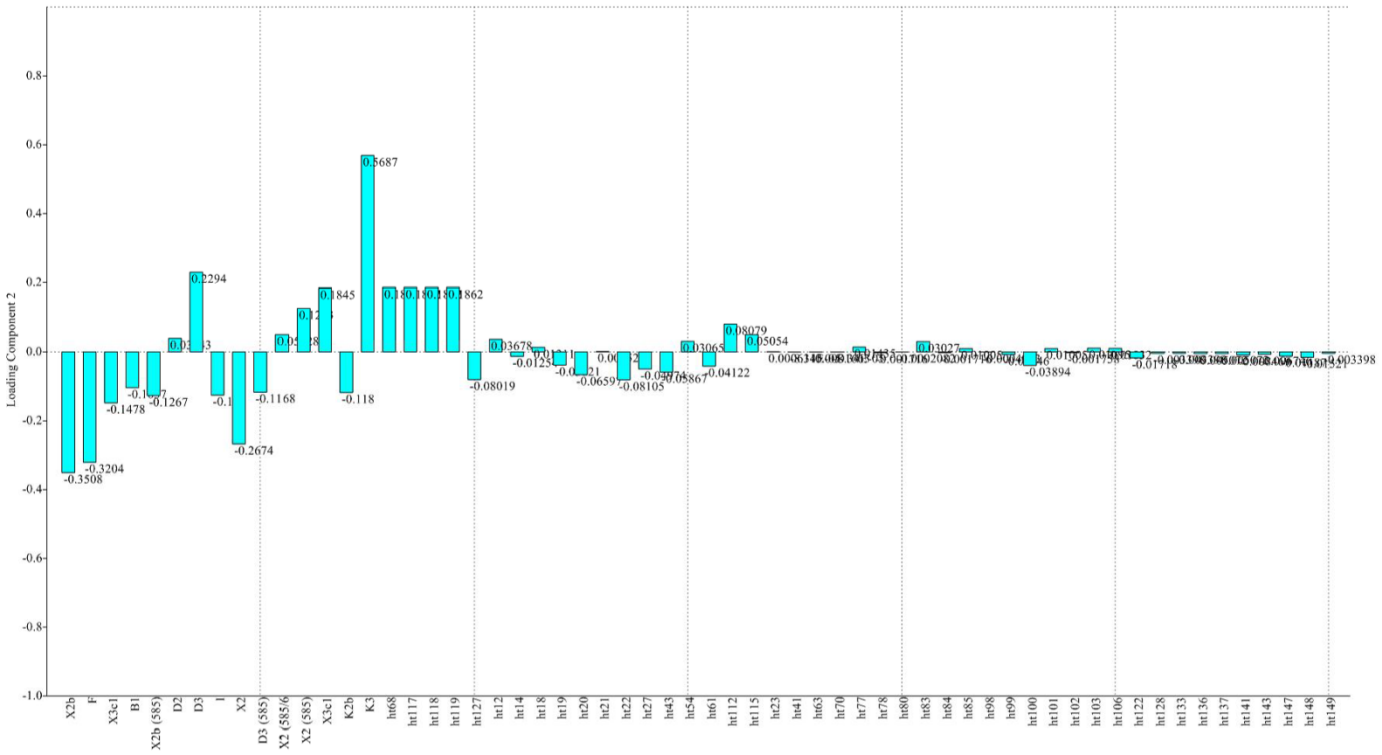


Figure S2: PCA plot based on relative mtDNA haplotype frequencies. The two dimensions display 48 % of the total variance. The contribution (loading) of each haplotype is presented by bar charts for component 1 and 2.

3. Additional outcome of thesis-related research

3.1 Schibler J, Elsner J, Schlumbaum A (2014) Incorporation of aurochs into a cattle herd in Neolithic Europe: single event or breeding? *Scientific Reports* 4. doi: 10.1038/Srep05798

SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
ARCHAEOLOGY
PALAEOECOLOGY
HAPLOTYPES

Received
17 March 2014

Accepted
4 July 2014

Published
23 July 2014

Correspondence and requests for materials should be addressed to A.S. (Angela.schlumbaum@unibas.ch)

Incorporation of aurochs into a cattle herd in Neolithic Europe: single event or breeding?

Jörg Schibler, Julia Elsner & Angela Schlumbaum

University of Basel, Department Environmental Science, Integrative Prehistory and Archaeological Science, Spalenring 145, CH 4055 Basel, Switzerland.

Domestication is an ongoing process continuously changing the lives of animals and humans and the environment. For the majority of European cattle (*Bos taurus*) genetic and archaeozoological evidence support initial domestication ca. 11'000 BP in the Near East from few founder aurochs (*Bos primigenius*) belonging to the mitochondrial DNA T macro-haplogroup. Gene flow between wild European aurochs of P haplogroup and domestic cattle of T haplogroup, coexisting over thousands of years, appears to have been sporadic. We report archaeozoological and ancient DNA evidence for the incorporation of wild stock into a domestic cattle herd from a Neolithic lake-dwelling in Switzerland. A complete metacarpus of a small and compact adult bovid is morphologically and genetically a female. With withers height of ca. 112 cm, it is comparable in size with small domestic cattle from contemporaneous sites in the area. The bone is directly dated to 3360–3090 cal BC and associated to the Horgen culture, a period of the secondary products revolution. The cow possessed a novel mtDNA P haplotype variant of the European aurochs. We argue this is either a single event or, based on osteological characteristics of the Horgen cattle, a rare instance of intentional breeding with female aurochs.

It is becoming increasingly clear, that the processes of domestication are more complex than previously thought[1–3]. Genetic signatures of these past human-animal relationships may be lost or blurred in modern livestock, but can be directly detected by ancient DNA analyses in a chronological and spatial setting[2,4–6]. The aurochs, roaming large parts of Eurasia and North Africa[7], was the largest animal to be domesticated with withers height between ca. 132 cm (small female) and 189 cm (large males)[8–10]. In the archaeological record, large and robust bones signify the presence of wild cattle, however, due to pronounced sexual dimorphism in bovids, morphological identification is ambiguous because small female aurochs overlap with robust male domestic cattle[8]. Moreover, the sizes of aurochs vary depending on geographical and chronological setting. Animals tend to be smaller in the southern regions and younger prehistoric periods of Europe[11]. Ancient DNA surveys of the maternally inherited mitochondrial DNA (mtDNA) of European aurochs revealed haplogroups P and E present in Northern/Central Europe[12,13], and aurochs of haplogroup T coexisting with P types in Italy[14,15]. Traces of female aurochs inheritance were detected in very few modern cattle through haplogroups P, R, Q[16]. Gene flow between wild and domestic cattle was a rare event[2]. The occasional archaeogenetic evidence of crossbreeding, namely the identification of morphological aurochs carrying the T haplotype, has been rejected on the plausible identification problems associated with fragmented remains[12]. Yet, the incorporation of wild animals into domestic stocks has been argued, for example, for pigs[2,17,18] and horses[19].

Results

Presented here are metric analyses and ancient DNA typing of mtDNA d-loop and allosomic zinc finger gene (zfx/zfy) of a complete left metacarpus from a Bovidae. The bone (Inv. Number 2048.1) is part of a large assemblage of slaughter or kitchen waste[8], excavated at Twann, Lake Biel, Switzerland (Twann Bahnhof), one of the famous Neolithic lake shore settlements in the Circum-Alpine area (Fig. 1, Supplementary note). It is associated with the uppermost layer (OH) of the Horgen culture, dated by dendrochronology to 3093–3072 BC[20]. The chronological position is confirmed by direct AMS-^{14}C-dating of the bone to 3360–3090 cal BC (ETH-42968: 94.5%)[21,22] (OxCal version v3.10).

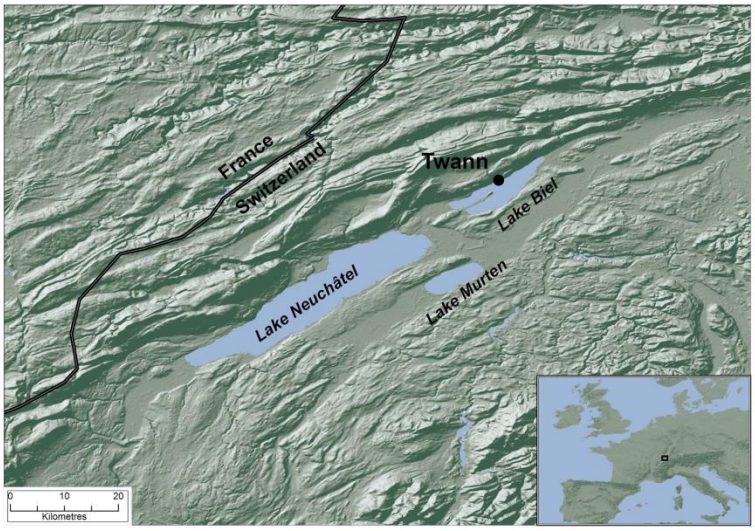


Figure 1 | **The site.** Location of Twann in Switzerland. Map was created with ArcGIS version 10 using SRTM data.

Archaeozoological status identification as small domestic cow. The bone belonged to an adult (older than 2 years) – the distal end is fully fused, the epiphyseal line is not visible – and does not show any pathologies (Fig. 2). The measurements taken according to von den Driesch[23] are: GL 184.9; Ll 181.1; Bp 52.6; SD 28.2; DD 17.4; Bd 52.2. These measurements are in accordance with an archaeozoological status assignment to fully domestic female cattle. The withers height calculated for the cow is 111.8 cm[24], which is the size of small modern cattle breeds such as the alpine Raetian Grey. A comparison of greatest length (GL) and distal breath (Bd) from complete cattle and aurochs metacarpi of contemporaneous sites in the area with metacarpus "2048.1" places the bone into the group of small domestic cattle (Fig. 3). Morphologically, the bone clearly lies outside the size of any known aurochs specimen. It should be noted, however, that findings of complete long bones are extremely rare in the archaeological record.

Genetic mtDNA haplotype and sex identification. A fragment of the mtDNA d-loop, diagnostic to distinguish between P and T maternal lineages in bovids, and nuclear zfx/zfy genes were PCR amplified and sequenced. The concatenated 361 bp mtDNA d-loop sequence displayed the characteristic substitutions of European aurochs P haplogroup and three further polymorphisms (pos. 16'074, 16'084, 16'085) in relation to the main P haplotype (DQ915577, Fig. 4, Supplementary Fig. S1). Sexing with zfy/zfx genes confirmed that the bone belonged to a female (Supplementary Fig. S1).

Discussion

Several scenarios may explain this finding. It may be argued that, similar to pigs, wild or domestic status in cattle cannot be reliably identified morphologically and that female aurochs size variability was much larger than expected. If this is true, miniature aurochs

Figure 2 | **The metacarpus sinister 2048.1 from Twann Bahnhof.** (A): front, (B): back, (C): after sampling for genetic analysis.

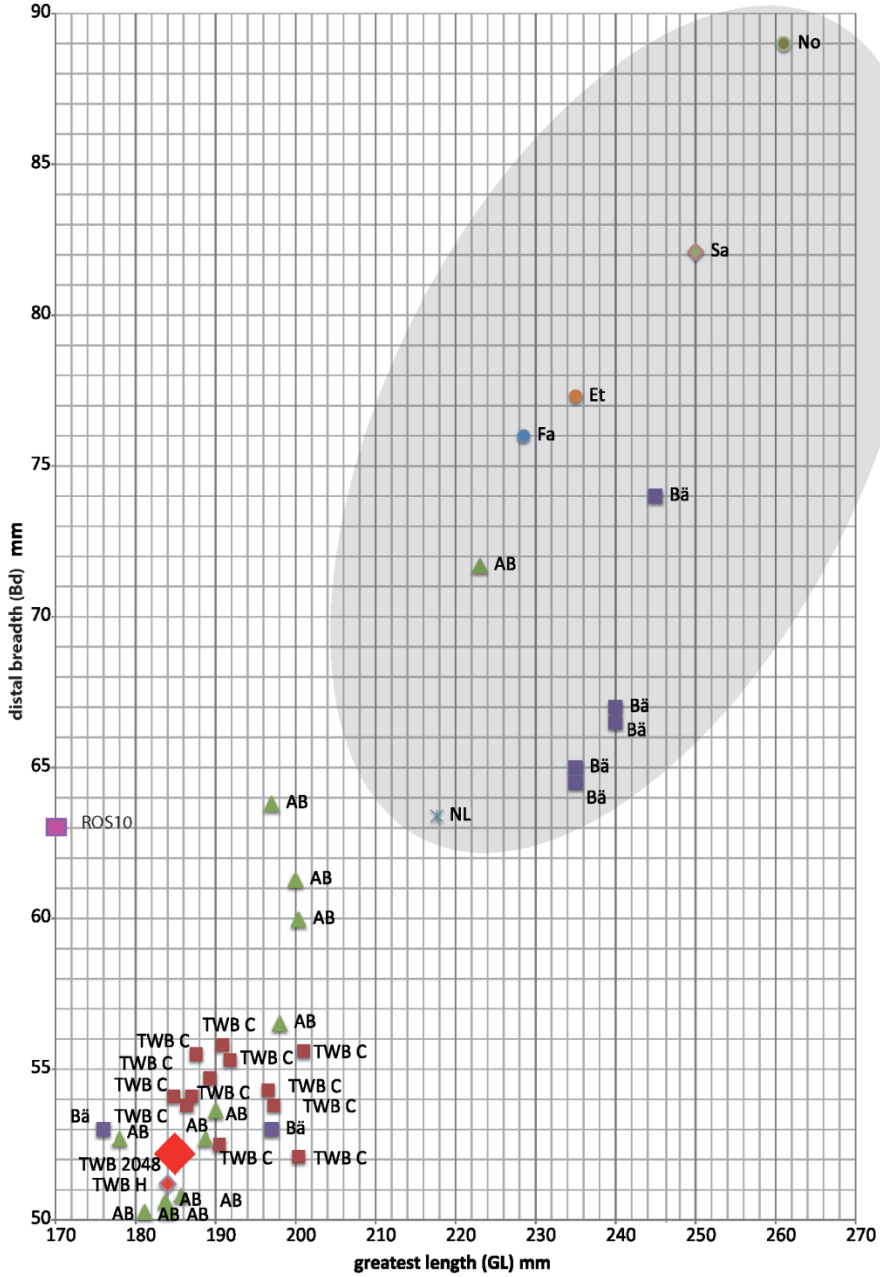


Figure 3 | Measurements of contemporary metacarpi from domestic and wild cattle from selected Swiss Neolithic sites and others. Greatest length (GL) and distal breath (Bd) of complete metacarpi from: AB = Arbon-Bleiche 3 (Horgen culture: 3384–3370 BC); TWB C = Twann (Cortaillod culture: 3838–3532 BC); TWB 2048; TWB H = Twann (Horgen culture 3360–3090 cal BC)[38]; BA = Burgäschisee-Süd (Cortaillod culture: 3820–3680 BC); Ros 10 = Rosenhof 10 (4770 ± 40 cal BC), not complete[13]; NL = Mesolithic aurochs River Tjonger valley[25]; Early/Late Neolithic aurochs[43] Et = Etival; Fa = Farges; Sa = Sauge, No = Novéant. Gray ellipse: morphological aurochs.

individuals or populations must have undetectably coexisted with large ones, a scenario we consider highly unlikely. Currently the smallest securely identified aurochs had 132 cm withers height (cited in[25]:), 20 cm larger than 2048.1 (Fig. 3).

Single events of gene flow between wild and domestic cattle sharing the same habitat at the same time is another possible explanation[26,27]. Yet genetic and morphometric evidence for crossbreeding in the archaeological record is controversial. Rare morphologically intermediate size types are often explained as crossbreeds, but the issue is disputed (e.g.[28–30]). The P lineage was previously identified in different skeletal elements from few bovids with ambiguous domestic status (Supplementary Table S2). Of these, the only one directly comparable to 2048.1 is the metacarpus Ros 10 belonging most probably to a small female aurochs (4770 ± 40 cal BC) (Fig. 3)[13]

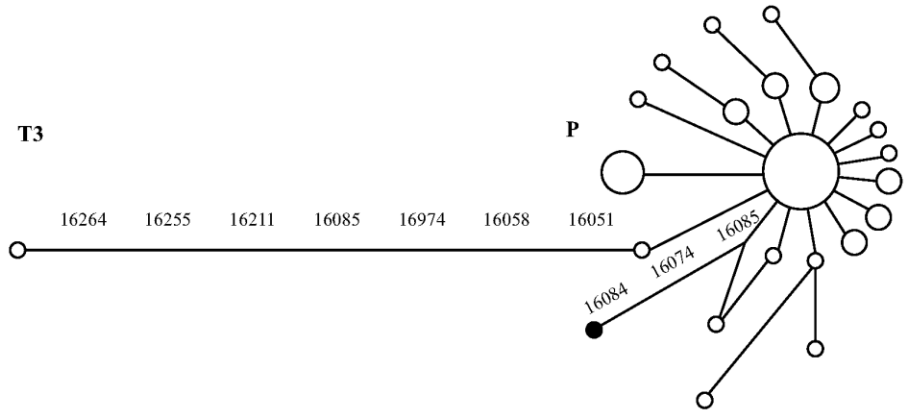


Figure 4 | Network comprising bovine P haplotypes. Median joining network of published mtDNA d-loop sequences (pos. 16'042–16'152 and 16'185–16'262 from V00654) from aurochs (Supplementary table S3). The position of the Twann metacarpus 2048.1 is indicated by a filled circle.

which is larger than 2048.1. Therefore, 2048.1 is definitely not of intermediate size. This also raises the question of how dramatic the height decrease of the offspring, in this case of domestic bull and a wild cow, in the first generation is. Chance events are even more unlikely considering both archaeozoological and -botanical data from the area suggesting that due to increasing human impact on the environment, local aurochs populations experienced greater competition for resources with domestic cattle and most probably withdrew to other areas[31,32].

Another, more likely explanation is repeated and intentional crossbreeding between the offspring of a female aurochs with domestic bulls over an extended time period, consequently keeping the females close to the settlement, starting in an unknown location sometimes before 3300 BC. Chance crossbreeding may have been the initial event but it was followed by capture and human interaction. Amongst others, this scenario was previously proposed for the bovine R lineage in modern Italian cattle[33]. Intentional breeding can lead to the appearance of domestic traits in a few generations, as has been shown for deer[34], and changes from wild to domestic appearance in cattle under management is suggested to be visible within 150–180 years[35]. It implies that early farmers took the opportunity to restock their cattle herds with wild aurochs. Such events appear to have been rare, as today no unambiguous domestic cattle with a P haplotype from the Mesolithic onwards has been found (Supplementary Tab. S2). However, the descendants of P type domestic cattle did survive in two known modern cattle[26] interpreted as descendants of a rare matrilinear introgression from wild aurochs[26,27].

The evidence for cattle improvement/control/regimen makes particular sense in light of what is known about the Horgen culture in Switzerland. During this cultural-time period (3400–2800 BC) cattle were smaller but more robust than in earlier and later Swiss Neolithic cultures[10,11]. First evidence of using cattle as draught animals and of intentional breeding of, particularly, cows during this period is discussed[10]. Withers heights vary between 102.5 and 132 cm and the appearance of the first castrated animals with withers height around 130 cm has been suggested[10]. The bones of hunted aurochs were never frequently encountered and their number decreases further during Neolithic times from the Cortaillod period onwards (c. 3900 BC)[32]. Therefore, incorporation of European aurochs into domestic herds is possibly related to improvements of domestic cattle during the period of the "secondary products revolution" in the 4th Millennium BC[36]. The contribution of human intention and selection on early domestic herds has been questioned recently[37], the Twann metarcarpus may, however, be evidence for genuine breeding during a time of innovation in animal husbandry and thus adds a further facet to the process of cattle domestication. It is probable that other, so far unnoticed, attempts also took place, as complete long bones are rarely found in the archaeological record and morphometric status identification of fragments is often difficult.

To further investigate the fate and legacy of P aurochs in domestic cattle, more ancient samples with unambiguous status identification need to be investigated. Furthermore, a genome will reveal the degree of domestication, phenotypic traits and other traits selected for in comparison to aurochs and modern cattle genomes. Together it might be even possible to clarify whether the Twann cow signifies an event of secondary domestication. Our results add to the growing body of novel insights from interdisciplinary research on early animal husbandry/management and the complex modes of human interaction with wild fauna.

Methods

Material. The Neolithic site of Twann – Bahnhof (Twann station) was excavated between 1975 and 1976 and today posesses UNESCO world heritage site status. In an excavated area of 2'300 m^2, 13 settlement phases were found. Ten phases belong to the Cortaillod culture and three to the Horgen (layer MH and OH) or Port-Conty culture (layer UH), specifying the transition from Cortaillod to Horgen cultures. Dendrochronological dates place the ten Cortaillod phases between 3838 and 3532 BC, and the three Horgen phases between 3405 and 3072 BC. All layers and archaeological material have been preserved in a waterlogged condition. The complete metacarpus 2048.1 was a chance find during collection of faunal remains to study DNA preservation in waterlogged material. It belongs to the layer OH (obere Horgener Schicht = upper Horgen culture layer). The identification as cattle (*Bos primigenius taurus*) was done by H.R. Stampfli[38]. Most other bone remains in this layer are fragmented and the assemblage is interpreted as slaughter and/or kitchen waste (Supplementary note).

DNA extraction, PCR, and sequencing. Ancient DNA work was carried out according to accepted standards in aDNA research and as established at IPAS, Basel, CH[4] and the Paleogenetics group in Mainz, D[39].

Basel: Bone preparation, DNA extraction and PCR amplification. The outer surface of the bone was removed with sandpaper and a cube of *c.* 1 cm^3 was cut with a Dremel® tool. The cube was ground with a mixer mill (Retsch MM2, Schieritz & Hauenstein, Allschwil, Switzerland). DNA extraction followed the User Developed Protocol: "Purification of total DNA from compact animal bone using the DNeasy® Blood & Tissue Kit" (Qiagen, Basel, Switzerland) for less than 100 mg. A mock control was performed with the sample. The extract was ultrapurified with water (molecular biology grade, Eppendorf, Allschwil, Switzerland) using 30 kD filter units (Amicon/Millipore, Zug, Switzerland). The final eluate was 200 µl.

Four partially overlapping targets of mitochondrial d-loop with different lengths covering nucleotide positions 15'903–16'312 (V00654) and two 27 bp segments of the zinc-finger genes in the X and Y chromosome (supplementary Table S1) were PCR amplified in 25 µl volumes containing 1.5 U AmpliTaq Gold, 1× GeneAmp PCR Gold buffer (150 mM Tris-HCl, 500 mM KCl, pH 8.0) and 2 mM $MgCl_2$ (all Applied Biosystems, Hombrechtikon, Switzerland); 0.4 mM dNTP Mix (Promega, Dübendorf, Switzerland); 0.2 mM of each primer; 20 µg/µl BSA (bovine serum

albumin, Roche, Basel, Switzerland), and 3–9 µl (5 µl for sexing PCRs) template DNA on a Mastercycler ProS (Eppendorf, Allschwil, Switzerland). The cycling conditions were: 12 min initial denaturation, followed by 50–70 cycles of denaturation at 95°C for 40 s, annealing at 50–58°C (55°C for sexing primers) for 30 s, and extension 72°C for 30 s, with a final extension of 60 s at 72°C. At least one non-template control was performed with all amplifications (Supplementary note). To overcome potential PCR inhibitors DNA extracts were diluted 1 : 10. MtDNA PCR products were cloned with the TOPO TA Cloning Kit (Invitrogen, Zug, Switzerland) following the manufacturer's protocol, except that the reaction volume was halved. One to four clones were sequenced and/or PCR products were directly sequenced by Microsynth (Balgach, Switzerland). Amplicons of the zinc finger loci were directly sequenced. All sequences obtained for 2048.1, either by cloning or by direct sequencing, are given in supplementary Fig. S1.

Sequences were aligned with BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html), using the *Bos taurus* reference genome V00654. A median joining network was built with NETWORK 4.6.1.2[40] using *Bos primigenius* sequence entries from Genbank (supplementary table S3). All sequences were truncated to positions 16'185–16'312 to include as many P type sequences as possible. A concatenated consensus sequence was deposited with Genbank (KJ101593).

Authenticity. Established standards in aDNA research at Integrative Prehistory and Archaeological Science (IPAS) were adhered to[4,41]. Briefly: all ancient DNA work (pre-PCR) was performed in dedicated, physically separated laboratories, following a strict one-way policy. Benches and tools were treated with bleach and UV irradiated, consumable plastic ware was UV-irradiated prior to use. No PCR products were observed in the negative controls (supplementary note). Each target was validated with two or three independent extractions and up to three PCR products.

From the site Twann Bahnhof several faunal remains were sampled and mtDNA preservation was confirmed (Supplementary note and Supplementary Tab. 4).

Mainz: DNA extraction, PCR, sequencing. The sample was processed in the ancient DNA facilities at the Institute of Anthropology, Mainz University (Germany), under strict rules for contamination prevention as described[39].

Four cubes of total 0.89 g were incubated in sodium hypochlorite solution (<5%) for 15 min and subsequently rinsed with water, which had been osmotically purified and UV-irradiated for 8 hours. After UV-irradiation of the dried bone cubes for 45 min from two sides, they were powdered using a mixer mill (MM200, Retsch).

DNA was extracted using phenol-chloroform-isoamylalcohol (25 : 24 : 1; Roth), washed and concentrated using 50 kDa 15 ml Amicons (Millipore). A blank control was processed during extraction.

PCR and Sanger sequencing were performed as described[42] using primers BosU8/BosL4 (5′- GTACATTATGTCAAATTCATTCTTGATAG- 3′/ 5′GATCCCTCTTCTCGCTCCG-3′) and BosU5/BosL5 (5′-TACCATGCCGCGTGAAACCA-3′/5′-TTCTTTCTTCAGGGCCATCTCA-3′). Amplicons cover positions 16'122 to 16'273 according to the reference sequence V00654. PCR blank controls were processed during each PCR run. In total eight PCR products per primer pair were successfully sequenced and used to create a consensus sequence by eye. Extraction and PCR blank controls did not show a band after PCR amplification.

1. Dobney, K. & Larson, G. Genetics and animal domestication: new windows on an elusive process. *J. Zool.* **269**, 261–271 (2006).
2. Larson, G. & Burger, J. A population genetics view of animal domestication. *Trends Genet.* **29**, 197–205, doi:10.1016/j.tig.2013.01.003. (2013).
3. Larson, G. *et al.* Current perspectives and the future of domestication studies. *Proc. Nat. Acad. Sci. U.S.A.* **111**, 6139–6146, doi:10.1073/pnas.1323964111 (2014).
4. Schlumbaum, A. *et al.* Ancient DNA, a Neolithic legging from the Swiss Alps and the early history of goat. *J. Archaeol. Sci.* **37**, 1247–1251, doi: 10.1016/j.jas.2009.12.025 (2010).
5. Larson, G. *et al.* Ancient DNA, pig domestication, and the spread of Neolithic into Europe. *Proc. Nat. Acad. Sci. U.S.A.* **104**, 15276–15281 (2007).
6. Zhang, H. *et al.* Morphological and genetic evidence for early Holocene cattle management in northeastern China. *Nat. Commun.* **4**, 2755, doi: 10.1038/ncomms3755 (2013).
7. Benecke, N. *Der Mensch und seine Haustiere.* 470 (Theiss, 1994).
8. Stampfli, H. R. Wisent, *Bison bonasus* (LINNÉ) 1758, Ur, *Bos primigenius* BOJANUS, 1827, und Hausrind *Bos taurus* (LINNÉ) 1758. In *Burgäschisee-Süd, Teil 3: Die Tierreste* Vol. 2 (eds Boesseneck, J., Jéquier, J.-P. & Stampfli, H. R.) 117–196 (Acta Bernensia, 1963).
9. Degerbøl, M. & Fredskild, B. The Urus (*Bos primigenius* Bojanus) and neolithic domesticated cattle (*Bos taurus* domesticus, LINNÉ) in Denmark. *Det Kongelige Danske Videnskabernes Selskab Biologiske Skrifter* **17**, 1–227 (1970).
10. Deschler-Erb, S. & Marti-Grädel, E. Viehhaltung und Jagd. Ergebnisse der Untersuchungen der handaufgelesenen Tierknochen. In *Die jungsteinzeitliche Siedlung Arbon Bleiche 3. Umwelt und Wirtschaft* Vol. 12 *Archäologie im Thurgau* (eds Jacomet, S., Leuzinger, U. & Schibler, J.) Ch. 3, 158–231 (Huber & Co AG, 2004).
11. Hüster-Plogmann, H. & Schibler, J. Archäozoologie. In *Ökonomie und Ökologie neolithischer und bronzezeitlicher Ufersiedlungen am Zürichsee Monographien der Kantonsarchäologie Zürich* (eds Schibler, J. *et al.*) Ch. 2, 40–121 (Direktion der öffentlichen Bauten des Kantons Zürich, Hochbauamt, Abteilung Kantonsarchäologie, 1997).
12. Edwards, C. J. *et al.* Mitochondrial DNA analysis shows a Near Eastern Neolithic origin for domestic cattle and no indication of domestication of European aurochs. *Proc. R. Soc. Lond. B* **274**, 1377–1385 (2007).
13. Scheu, A. *et al.* Ancient DNA provides no evidence for independent domestication of cattle in Mesolithic Rosenhof, Northern Germany. *J. Archaeol. Sci.* **35**, 1257–1264 (2008).
14. Mona, S. *et al.* Population dynamics of the extinct European aurochs: genetic evidence of a north-south differentiation pattern and no evidence of post-glacial expansion. *BMC Evol. Biol.* **10**, 83 (2010).
15. Lari, M. *et al.* The complete mitochondrial genome of an 11,450 year-old aurochsen (Bos primigenius) from Central Italy. *BMC Evol. Biol.* **11**, 32 (2011).
16. Achilli, A. *et al.* The multifaceted origin of taurine cattle reflected by the mitochondrial genome. *PLoS ONE* **4**, e5753, doi: 10.1371/journal.pone.0005753 (2009).
17. Ottoni, C. *et al.* Pig Domestication and Human-Mediated Dispersal in Western Eurasia Revealed through Ancient DNA and Geometric Morphometrics. *Mol. Biol. Evol.* **30**, 824–832, doi:10.1093/molbev/mss261 (2013).
18. Scheu, A., Geörg, C., Schulz, A., Burger, J. & Benecke, N. in *Population Dynamics in Prehistory and Early History - New Approaches Using Stable Isotopes and Genetics TOPOI 5* (eds Kaiser, E., Burger, J. & Schier, W.) 45–54 (De Gruyter, 2012).
19. Warmuth, V. *et al.* Reconstructing the origin and spread of horse domestication in the Eurasian steppe. *Proc. Nat. Acad. Sci. U.S.A.* **109**, 8202–8206, doi:10.1073/pnas.1111122109 (2012).
20. Stöckli, W. E., Niffeler, U. & Gross-Klee, E. Neolithikum. In *Die Schweiz vom Paläolithikum bis zum frühen Mittelalter* Vol. 2 358 (Schweizerische Gesellschaft für Ur- und Frühgeschichte, Basel, 1995).
21. Reimer, P. J. *et al.* IntCal09 and Marine09 Radiocarbon Age Calibration Curves, 0–50,000 Years cal BP. *Radiocarbon* **51**, 1111–1150 (2009).
22. Bronk Ramsey, C. Development of the radiocarbon calibration program OxCal. *Radiocarbon* **43**, 1029–1058 (2001).
23. von den Driesch, A. *A guide to the measurement of animal bones from archaeological sites.* Vol. 1 (Harvard University, 1976).
24. Matolcsi, J. Historische Erforschung der Körpergrösse des Rindes auf Grund von ungarischem Knochenmaterial. *J. Anim. Breed. Genet.* **87**, 89–137 (1970).
25. Prummel, W. & Niekus, M. J. L. T. Late Mesolithic hunting of a small female aurochs in the valley of the River Tjonger (the Netherlands) in the light of Mesolithic aurochs hunting in NW Europe. *J. Archaeol. Sci.* **38**, 1456–1467, doi:10.1016/j.jas.2011.02.009 (2011).
26. Stock, F. *et al. Cytochrome b* sequences of ancient cattle and wild ox support phylogenetic complexity in the ancient and modern bovine populations. *Anim. Gen.* **40**, 694–700, doi:10.1111/j.1365-2052.2009.01905.x (2009).
27. Achilli, A. *et al.* Mitochondrial genomes of extinct aurochs survive in domestic cattle. *Curr. Biol.* **18**, 158 (2008).
28. Kyselý, R. Aurochs and potential crossbreeding with domestic cattle in Central Europe in the Eneolithic period. A metric analysis of bones from the archaeological site of Kutná Hora-Denemark (Czech Republic). *Anthropozoologica* **43**, 7–37 (2008).
29. Götherström, A. *et al.* Cattle domestication in the Near East was followed by hybridization with aurochs bulls in Europe. *Proc. R. Soc. Lond. B* **272**, 2345–2350 (2005).
30. Bollongino, R., Elsner, J., Vigne, J.-D. & Burger, D. Y-SNPs do not indicate hybridization between European aurox and domestic cattle. *PLoS One* **3**, e3418, doi:10.1371/journal.pone.0003418 (2008).
31. Hüster-Plogmann, H., Schibler, J. & Jacomet, S. The significance of aurochs as a hunted animal in the Swiss Neolithic. *Schriftenreihe des Neanderthalmuseums, Mettmann* 151–160 (1999).
32. Schibler, J. & Steppan, K.-H. Human impact on the habitat of large herbivores in Eastern Switzerland and South West Germany in the Neolithic. *Archaeofauna* **8**, 87–99 (1999).
33. Bonfiglio, S. *et al.* The enigmatic origin of bovine mtDNA haplogroup R: sporadic interbreeding or an independent event of (*Bos primigenius*) domestication in Italy? *PLoS One* **5**, e15760, doi:10.1371/journal.pone.0015760 (2010).
34. Hemmer, H. Neumühle-Riswicker Hirsche: Erste planmässige Zucht einer neuen Nutztierform. *Naturwiss. Rundsch.* **58**, 255–261 (2005).
35. Gautier, A. *La domestication: et l'homme créa ses animaux.* (Errance, 1990).
36. Sherratt, A. in *Pattern of the Past: Studies in honour of David Clarke* (eds Hodder, I., Isaac, G. & Hammond, N.) 261–305 (Cambridge University Press, 1981).
37. Marshall, F. B., Dobney, K., Denham, T. & Capriles, J. M. Evaluating the roles of directed breeding and gene flow in animal domestication. *Proc. Nat. Acad. Sci. U.S.A.*, doi:10.1073/pnas.1312984110 (2014).
38. Stampfli, H. R. Tierknochenfunde. In *Die Siedlungsreste der Horgener Kultur* Vol. 7 *Die Neolithischen Ufersiedlungen von Twann* (ed Furger, A. R.) 141–160 (Staatlicher Lehrmittelverlag, 1980).
39. Bramanti, B. *et al.* Genetic discontinuity between local hunter-gatherers and Central Europe's first farmers. *Science* **326**, 137–140 (2009).
40. Bandelt, H.-J., Macaulay, V. & Richards, M. Median networks: Speedy construction and greedy reduction, one simulation, and two case studies from human mtDNA. *Mol. Phylogenet. Evol.* **16**, 8–28 (2000).
41. Gilbert, M. T. P., Bandelt, H.-J., Hofreiter, M. & Barnes, I. Assessing ancient DNA studies. *Trends Ecol. Evol.* **20**, 541–544 (2005).

42. Scheu, A. *Palaeogenetische Studien zur Populationsgeschichte von Rind und Ziege mit einem Schwerpunkt auf dem Neolithikum in Südosteuropa.* Vol. 4 (Marie Leidorf GmbH, 2012).
43. Chaix, L. & Arbogast, R. M. Holocene aurochs from Western Europe: osteometrical data. In *Archaeology and Biology of the Aurochs* Vol. 1 (ed Weniger, G.-C.) 35–48 (Neanderthal Museum, 1999).

Acknowledgments

We thank the Archaeological Service of the Kanton Bern ADB for the sample. We thank Amelie Scheu for performing the independent verification and José Granado for help with lab work. We are grateful to Greger Larson and Keith Dobney for critical comments on the manuscript, and Ben Jennings for improving our English. The project was funded by Swiss National Science Foundation, project CR13/1-140638, and the University of Basel.

Author contributions

J.S. and A.S. conceived the work, J.S. performed measurements and archaeozoological work, J.E. performed aDNA analysis, A.S. supervised work. A.S., J.S., J.E. wrote the paper, all authors approved the final version of the paper.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Schibler, J., Elsner, J. & Schlumbaum, A. Incorporation of aurochs into a cattle herd in Neolithic Europe: single event or breeding? *Sci. Rep.* **4**, 5798; DOI:10.1038/srep05798 (2014).

Electronic supplementary material

Supplementary information

Incorporation of aurochs into a cattle herd in Neolithic Europe: single event or breeding?

Jörg Schibler[1], Julia Elsner[1], Angela Schlumbaum[1]

[1] University of Basel, Department Environmental Science, Institute of Prehistory and Archaeological Science

Supplementary figures

Figure S1. Alignment of concatenated sequences obtained by cloning or direct sequencing of mtDNA d-loop (a) and Zfx genes (b)

Supplementary tables

Table S1. List of primer sequences for mtDNA targets and zinc finger genes[1,2] used in Basel

Table S2. Potentially domestic cattle with P haplotype[1,3-6]

Table S3. List of *Bos primigenius* accessions used for network[1,4,7-11]

Table S4. Results from mtDNA d-loop typing from faunal remains of the site Twann Bahnhof

Supplementary note

Archaeology and archaeozoology

Twann (429 m asl, 47° 5′ 40.92″ N, 7° 9′ 22.32″ E) is situated at the northern shore of the lake Bienne, 8.5 km southwest of Biel/Bienne in Switzerland. Organic material like plant remains (seeds, fruits, wood) and bones are preserved in good quality due to the waterlogged deposition that is typical for Neolithic and Bronze Age lakeshore settlements. In total 246'226 ceramic fragments, 92'573 animal bone fragments, 9'372 antler artifacts, 7'265 bone artifacts, 3'525 rock artifacts and 2'923 flint artifacts were found (for the site [12], for the layers of the Horgen culture [13]). All bones are stored at Archaeological Services of the canton Bern.

The preservation of complete long bones is generally extremely rare. For example, in the Horgen layers of Twann 736 cattle remains were found, only two of them were complete metacarpi [14]. As thus comparisons of measurements and calculation of wither heights with other cattle from this time in Europe is restricted. However, based on measurements of cattle phalanx 1 it is known, that cattle were smaller and more robust during Horgen time than before and after, indicating a different use of these animals, and that people had the knowledge about breeding these animals [15].

The faunal remains from the Horgen layers at Twann are dominated by domestic animals (UH=lower Horgen culture layer: 83.0%; MH=middle Horgen culture layer: 90.4%; OH=upper Horgen culture layer: 84.1%. Basis: number of identifiable bone fragments). In the upper two layers pig (*Sus domesticus*) is the most important species on the basis of fragment numbers and calculated minimal numbers of individuals. Pig being the most important domestic species is a well-known phenomenon in the Horgen culture all over Switzerland [16]. Concerning Bovids, domestic cattle (25% of total remains) and few aurochs (0.2% of total remains) were identified. Hunting appears to be of little importance during this time period, because only few remains of wild animals were found. They are mostly red deer (*Cervus elaphus*) [14].

For archaeogenetic analyses "2048.1" was not processed alone but together with bovids from the same site and other sites. For Twann Bahnhof horse and bovid remains were analyzed by PCR (Table S4). One mock extraction control and at least one PCR blank per 8 samples were performed. For the Twann bovids no bands were found in the negative controls, whereas for the horse samples bands in negative controls were either bacteria, fungi or unidentifiable in rare instances. From the 30 bovid samples only 5 were successful and the only *Bos taurus* belonged to the T3 haplogroup (Table S4).

The nomenclature used is according to International Commission on Zoological Nomenclature 2003. It is noted that alternatively European aurochs is *Bos primigenius ssp. primigenius* and domestic cattle is *Bos primigenius* ssp. *taurus*.

Supplementary references

1 Scheu, A. *et al.* Ancient DNA provides no evidence for independent domestication of cattle in Mesolithic Rosenhof, Northern Germany. *J. Archaeol. Sci* **35**, 1257-1264 (2008).

2 Bollongino, R. Die Herkunft der Hausrinder in Europa. Eine aDNA-Studie an neolithischen Knochenfunden, Mainz, (2005).

3 Anderung, C. *et al.* Prehistoric contacts over the Straits of Gibraltar indicated by genetic analysis of Iberian Bronze Age cattle. *Proc. Nat. Acad. Sci. U.S.A.* **102**, 8431-8435 (2005).

4 Edwards, C. J. *et al.* Mitochondrial DNA analysis shows a Near Eastern Neolithic origin for domestic cattle and no indication of domestication of European aurochs. *Proc. R. Soc. Lond. B* **274**, 1377-1385 (2007).

5 Stock, F. *et al. Cytochrome b* sequences of ancient cattle and wild ox support phylogenetic complexity in the ancient and modern bovine populations. *Anim. Gen.* **40**, 694-700, doi:10.1111/j.1365-2052.2009.01905.x (2009).

6 Bollongino, R., Edwards, C. J., Burger, J., Alt, K. W. & Bradley, D. G. Early history of European domestic cattle as revealed by ancient DNA. *Biol. Lett.* **2**, 155-159 (2006).

7 Bollongino, R., Elsner, J., Vigne, J.-D. & Burger, D. Y-SNPs do not indicate hybridization between European aurox and domestic cattle. *PLoS One* **3**, e3418, doi:10.1371/journal.pone.0003418 (2008).

8 Gravlund, P. *et al.* Ancient DNA extracted from Danish aurochs (Bos primigenius): Genetic diversity and preservation. *Ann. Anat.* **194**, 103-111, doi:http://dx.doi.org/10.1016/j.aanat.2011.10.011 (2012).

9 Troy, C. S. *et al.* Genetic evidence for Near-Eastern origins of European cattle. *Nature* **410**, 1088-1091. (2001).

10 Pruvost, M. *et al.* Freshly excavated fossil bones are best for amplification of ancient DNA. *Proc. Nat. Acad. Sci. U.S.A.* **104**, 739-744 (2007).

11 Zeyland, J. *et al.* Complete mitochondrial genome of wild aurochs (Bos primigenius) reconstructed from ancient DNA. *Pol. J. Vet. Sci.* **16**, 265-273 (2013).

12 Stöckli, W. E., Niffeler, U. & Gross-Klee, E. Neolithikum. In *Die Schweiz vom Paläolithikum bis zum frühen Mittelalter* Vol. 2 358 (Schweizerische Gesellschaft für Ur- und Frühgeschichte, Basel, 1995).

13 Furger, A. R. *Die Siedlungsreste der Horgener Kultur.* Vol. 7 (Staatlicher Lehrmittelverlag Bern, 1980).

14 Stampfli, H. R. Tierknochenfunde. In *Die Siedlungsreste der Horgener Kultur* Vol. 7 *Die Neolithischen Ufersiedlungen von Twann* (ed A.R. Furger) 141-160 (Staatlicher Lehrmittelverlag, 1980).

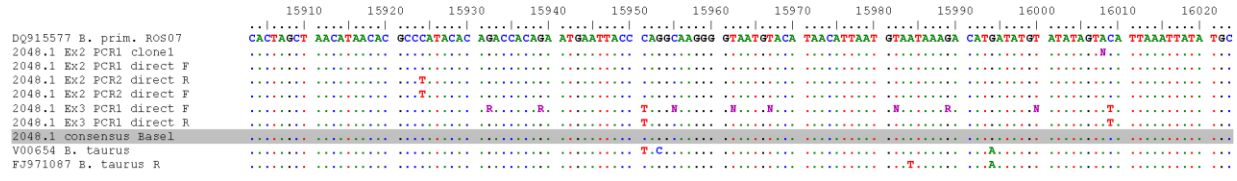
15 Hüster-Plogmann, H. & Schibler, J. Archäozzologie. In *Ökonomie und Ökologie neolithischer und bronzezeitlicher Ufersiedlungen am Zürichsee Monographien der Kantonsarchäologie Zürich* (eds J. Schibler *et al.*) Ch. 2, 40-121 (Direktion der öffentlichen Bauten des Kantons Zürich, Hochbauamt, Abteilung Kantonsarchäologie, 1997).

16 Schibler, J. The economy and environment of the 4th and 3rd millennia BC in the northern Alpine foreland based on studies of animal bones. *Environmental Archaeology* **11**, 49-64 (2006).

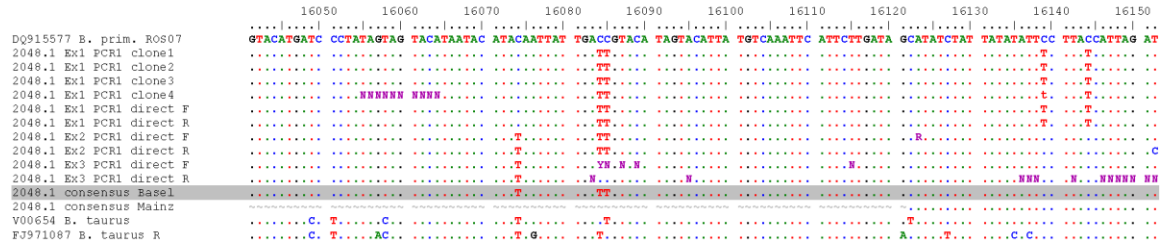
Supplementary Fig S1: Alignment of concatenated sequences obtained by cloning or direct sequencing of mtDNA d-loop (a) and Zfx genes (b)

(a)

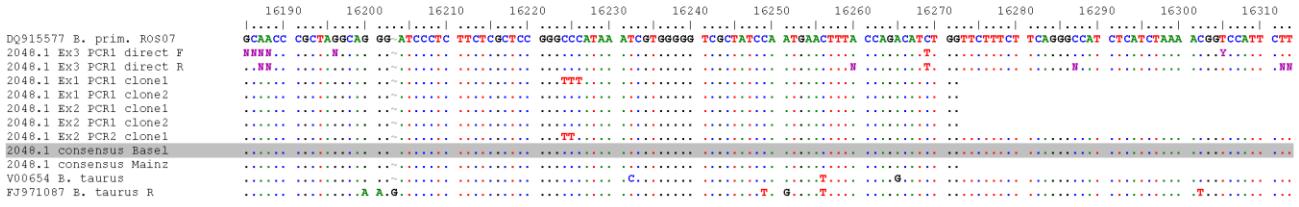
H1 - L2



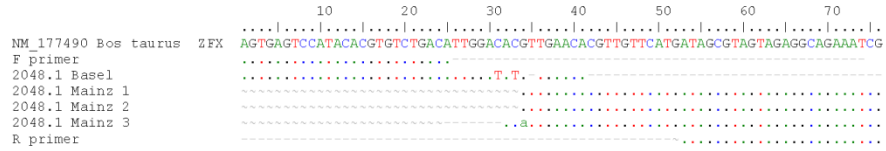
H3 - L4



H5 - L6/L7



(b) ZFX



Supplementary Tab. S1: List of primer sequences for mtDNA targets and zinc finger genes [1, 2] used in Basel

Name	Locus	Sequence 5'-3'	Position of amplicon	Length of amplicon
H1	HVR1, mitochondrial d-loop (V00654)	gca ccc taa cca aat att aca aac ac	15'903-16'023	121 bp
L2		gtc atg tac ttg ctt ata tgc atg gg		
H3		tat gcc cca tgc ata taa gca a	16'041-16'152	112 bp
L4		cgg cat ggt aat taa gct cgt g		
H5		tac cat gcc gcg tga aac ca	H5-L6: 16'185-16'271; H5-L7: 16'185-16'312	85 bp 128 bp
L6		tga gat ggc cct gaa gaa aga a		
L7		tcc atc gag atg tct tat tta aga gga		
Zfx3L	Zinc finger, X chromosome (NM_177490)	agt gag tcc ata cac gtg tct gac a	459-486	27 bp
Zfx3U		cga ttt ctg cct cta cta cgc tat		
Zfy3L	Zinc finger, Y chromosome (NM_177491)	cct cta cta cac tac cat gaa caa t	424-450	27 bp
Zfy3U		gtc ttg acc agt gag tct gta cat		

Supplementary Tab 2. Potentially domestic cattle with P haplotype															
sample	Genbank acc. No	element	morphological identification	mtDNA target	haplogroup	sex	site	country	date	reference	remark				
KER5	EF563959	1st phalanx	Bt	d-loop	P	nd	Kernavé	Lithuania	13 - 14. cent AD	Anderung et al unpublished	collected as primigenius, database entry wrong				
KR5	EF563958	tooth, not known which	Bt	d-loop	P	nd	Kretuonas	Lithuania	middle neolithic	Anderung et al unpublished	collected as primigenius, database entry wrong				
S33	EF563943	lower molar	Bt/bovid	d-loop	P	nd	Portalon Atapuerca	Spain	Bronze Age, 6165 +/-45	Anderung et al unpublished	collected as primigenius, database entry wrong				
SVO3	DQ915571	not given	Bt	d-loop	P	male	Svodin	Slowakia	3000 BC, Lengyel	Bollongino et al. 2006, Edwards et al 2007	possible error in identification, or introgression, too fragmentory for classification				
SVO3	FJ392912	not given	Bp/Bt, as BP in Genbank	cytochrome b	P	male	Svodin	Slowakia	3000 BC, Lengyel	Stock et al 2009	no clear classification				
MAD15	AY847197	permanent upper left premolar	Bt	d-loop	P	nd	Portalon Atapuerca	Spain	Bronze Age 1740 cal BC	Anderung et al. 2005	argue that it is a wild animal				
Ros 10	DQ915567	metacarpus	Bt/Bp?	d-loop	P	female	Rosenhof	Germany	4770 ± 40 cal BC	Scheu et al 2007	argue that it is a wild animal				
Ros 1	DQ915561	metatarsus	Bt/Bp?	d-loop	P	female	Rosenhof	Germany	4840 ± 80 cal BC	Scheu et al 2007	argue that it is a wild animal				
Ros 11	DQ915568	premaxille	Bt/Bp?	d-loop	P	female	Rosenhof	Germany	Late Mesolithic/Early Neolithic	Scheu et al 2007	argue that it is a wild animal				
Ros 12	DQ915569	3rd phalanx	Bt/Bp?	d-loop	P	female	Rosenhof	Germany	Late Mesolithic/Early Neolithic	Scheu et al 2007	argue that it is a wild animal				

Supplementary Table S3. List of *Bos primigenius* accessions used for network

Sample	site	country	date	Genbank acc. no.	reference
TBW44	Twann-Bahnhof	Switzerland	Neolithic	KJ101593	this paper
CPC-3	Carsington Pasture Cave	Britain	Mesolithic/Neolithic	DQ915526	Edwards et al. 2007
CPC-5	Carsington Pasture Cave	Britain	Mesolithic/Neolithic	DQ915528	Edwards et al. 2007
CPC-13	Carsington Pasture Cave	Britain	Mesolithic/Neolithic	DQ915535	Edwards et al. 2007
EIL6	Eilsleben	Eastern Germany	Earliest LBK, c. 5000 BC	DQ915542	Edwards et al. 2007
EIL14	Eilsleben	Eastern Germany	Earliest LBK, c. 5000 BC	DQ915543	Edwards et al. 2007
ETI1	Etival	France	Mesolithic, 5464+-78 BC	DQ915544	Edwards et al. 2007
GL418	Friebritz	Austria	middle Neolithic, c. 4900-4700 BC	DQ915548	Edwards et al. 2007
LJU3	Mala Triglavca	Slovenia	Mesolithic, c. 7000 BC	DQ915554	Edwards et al. 2007
NES2	Neustadt Holstein	Northern Germany	c. 4500-4100 BC	DQ915557	Edwards et al. 2007
ROS1	Rosenhof	Northern Germany	late Mesolithic/early Neolithic	DQ915561	Edwards et al. 2007
ROS2	Rosenhof	Northern Germany	late Mesolithic/early Neolithic	DQ915562	Edwards et al. 2007
ROS4	Rosenhof	Northern Germany	late Mesolithic/early Neolithic	DQ915564	Edwards et al. 2007
ROS5	Rosenhof	Northern Germany	late Mesolithic/early Neolithic	DQ915565	Edwards et al. 2007
ROS7	Rosenhof	Northern Germany	late Mesolithic/early Neolithic	DQ915566	Edwards et al. 2007
ROS10	Rosenhof	Northern Germany	late Mesolithic/early Neolithic	DQ915567	Edwards et al. 2007
ROS11	Rosenhof	Northern Germany	late Mesolithic/early Neolithic	DQ915568	Edwards et al. 2007
SVO3	Svodin	Slovakia	Lengyel, late Neolithic, c. 3000 BC	DQ915571	Edwards et al. 2007
SZE2	Szegvár-Tüzköves	Hungary	Neolithic, c. 5500-5000 BC	DQ915573	Edwards et al. 2007
NES3	Neustadt Holstein	Northern Germany	c. 4500-4100 BC	KC172646	Scheu et al. unpublished, submitted 2012
TP65	Totty Pot	Britain	7570-7320 cal BP	DQ915574	Edwards et al. 2007
TP65	Totty Pot	Britain	7570-7320 cal BP	AF336746	Troy et al. 2001
ZMK 58/2002	Bimpel	Denmark	2945+-25 KIA-19279 BP	JQ269329	Gravlund et al. 2012
ZMK 17/1863	Tinglev Sø	Denmark	3600+-35 Ua-21580 BP	JQ269326	Gravlund et al. 2012
ZMK 83/1942	Grænge	Denmark	9830+-60 Ua-21577 BP	JQ269325	Gravlund et al. 2012
KER5	Kernavé	Lithuania	13.- 14. cent AD	EF563959	Anderung et al. unpublished, submitted 2007
Hun-BPr	Polgar-Csoszhalom	Hungary	late Neolithic	EF362615	Priskin et al. unpublished 2007
MAD15	Portalo´ n, Atapuerca	Spain	1740 cal B.C.	AY847197	Anderung et al. 2005
PVL04	Pontvallain, La Sarthe	France	3200 BP	EF187280	Pruvost et al. 2007
ZMK 58/2002	Bimpel	Denmark	2865+-35 Ua-32310 BP	JQ269328	Gravlund et al. 2012
CPC-11	Carsington Pasture Cave	Britain	Mesolithic/Neolithic	DQ915533	Edwards et al. 2007
CPC-12	Carsington Pasture Cave	Britain	Mesolithic/Neolithic	DQ915534	Edwards et al. 2007
CPC-2	Carsington Pasture Cave	Britain	Mesolithic/Neolithic	DQ915525	Edwards et al. 2007
PWA	Pisz, Pisz Forest	Poland	1500 BP	JQ437479	Zeyland et al. 2013
CHWF	Charterhouse Warren Farm Swallet	Britain	4090-3720 cal. BP	DQ915524	Edwards et al. 2007
CHWF	Charterhouse Warren Farm Swallet	Britain	4090-3720 cal. BP	AF336745	Troy et al. 2001
ALB2	Albertfalva	Hungary	Bell Beaker, early Bronze Age	DQ915519	Edwards et al. 2007
ALB4	Albertfalva	Hungary	Bell Beaker, early Bronze Age	DQ915521	Edwards et al. 2007
CAT1	Cave a l'Ours	France	c. 3694 cal. BC	DQ915523	Edwards et al. 2007
KR5	Kretuonas	Lithuania	middle neolithic	EF563958	Anderung et al. unpublished, submitted 2007
CPC-6	Carsington Pasture Cave	Britain	Mesolithic/Neolithic	DQ915529	Edwards et al. 2007
CPC-7	Carsington Pasture Cave	Britain	Mesolithic/Neolithic	DQ915530	Edwards et al. 2007
CPC-8	Carsington Pasture Cave	Britain	Mesolithic/Neolithic	DQ915531	Edwards et al. 2007
CPC-14	Carsington Pasture Cave	Britain	Mesolithic/Neolithic	DQ915536	Edwards et al. 2007
ZMK 11/1937	Bellinge	Denmark	8460+-55 Ua-21572 BP	JQ269327	Gravlund et al. 2012
NORF	North Ferriby	Britain	3990-3720 cal. BP	DQ915558	Edwards et al. 2007
NORF	North Ferriby	Britain	3990-3720 cal. BP	AF336747	Troy et al. 2001
D740	Bob's Cave	Britain	12380-12200 cal. BP	DQ915538	Edwards et al. 2007
ZMK 43/1996	Tangelsbjerg	Denmark	9810+-60 Ua-21578	JQ269331	Gravlund et al. 2012
H3	Ecsegfalva 23	Hungary	early Neolithic, c. 5900-5500 BC	DQ915550	Edwards et al. 2007
CPC98	Carsington Pasture Cave	Britain	Mesolithic/Neolithic	DQ915537	Edwards et al. 2007
CPC98	Carsington Pasture Cave	Britain	Mesolithic/Neolithic	AF336748	Troy et al. 2001
NES1	Neustadt Holstein	Northern Germany	c. 4500-4100 BC	DQ915556	Edwards et al. 2007
PAR1	Grotte du Gardon	France	c. 3340-3150 cal. B.C.	DQ915560	Edwards et al. 2007
ROS12	Rosenhof	Northern Germany	late Mesolithic/early Neolithic	DQ915569	Edwards et al. 2007
NES7	Neustadt Holstein	Northern Germany	c. 4500-4100 BC	KC172650	Scheu et al. unpublished, submitted 2012
ZMK 10/1937	Barritskov	Denmark	9410+-60 Ua-21571 BP	JQ269330	Gravlund et al. 2012
EIL5	Eilsleben	Eastern Germany	Earliest LBK, c. 5000 BC	DQ915541	Edwards et al. 2007
ZMK 18/1903	Brabrand Sø	Denmark	9410+-60Ua-21571 BP	JQ269333	Gravlund et al. 2012
HAL1	Halle	Eastern Germany	c. 100 BC Iron Age	DQ915552	Edwards et al. 2007
ROS3	Rosenhof	Northern Germany	late Mesolithic/early Neolithic	DQ915563	Edwards et al. 2007
ZMK 10/1981	Kainsbakke	Denmark	4060+-50 Ua-24709 BP	JQ269334	Gravlund et al. 2012
ALL1	Allendorf	Eastern Germany	12,082-11,978 cal. B.P.	DQ915522	Edwards et al. 2007
ZMK 3/1893	Åmølle	Denmark	5855+-50 Ua-23538 BP	JQ269332	Gravlund et al. 2012
S33	Portalon Atapuerca	Spain	Bronze Age, 6165 +/-45	EF563943	Anderung et al. unpublished, submitted 2007
ROU6	Roucadour	France	Chasseen C2a	FJ005307	Bollongino et al. 2008
WAN5	Wangels	Germany	6000-3500 calBP, TBK	JX861237	Scheu et al. 2008

Supplementary Tab 4: Results from mtDNA d-loop typing from faunal remains of the site Twann Bahnhof

Laboratory Nur	Archaeological Code	Morphological Species De	Skeletal Element	mtDNA amplif	Genetic Species Determination
TWB2	1206.516	Equus	Phalanx I	no	
TWB3	1009.6	Equus	Metatarsus III dext dist	**yes**	*Equus caballus*
TWB4	167	Equus	Phalanx I post dext	**yes**	*Equus caballus*
			Humerus prox epiphysis sin	n/a	
			Phalanx II	n/a	
TWB5	909.21 Layer E6	Equus	Phalanx I ant sin	**yes**	*Equus caballus*
TWB6	148	Equus	Metacarpus III dext dist	no	
			Humerus sin distal	no	
			Phalanx III sin post?	n/a	
TWB7	168	Equus	Pelvis sin	no	
TWB8	921.13 Layer E5	Equus	Pelvis sin	no	
TWB9	1723.5	Equus	Pd2 inf max dext	**yes**	*Equus caballus*
TWB10	1247 V-10	Equus	Scapula sin	**yes**	*Equus caballus*
TWB11	1109.7	Equus	Metatarsus sin prox	no	
TWB12	1116.5	Equus	Sesamoid	no	
TWB13	1127 V-10	Equus	Sesamoid	**yes**	*Equus caballus*
TWB14	1120.6	Equus	Phalanx III dext	no	
TWB15	937.12	Equus	Pelvis sin	no	
TWB16	1815 VIII	Equus	Metatarsus prox dext	no	
TWB17	1044.6	Equus	Hamatum (Carpalia) dext	no	
TWB18	169	Equus	Metapodial, fragmented	no	
			Metacarpus IV sin dist	no	
TWB19	895.6	Equus	Undetermined	no	
TWB20	916.8	Equus	Tibia pox dext	no	
TWB21	339	Equus	Radius sin prox	no	
TWB22	761.15	Equus/Cervus/Bos?	Tibia?, fragmented	no	
TWB23	1074.3	Equus/Bos?	Femur distal sin	**yes**	*Alces alces*
TWB24a	1215.5 Layer OS9	Bos taurus	Mandibular tooth	**yes**	*Bos taurus*
TWB24b	1215.5 Layer OS9	Bos taurus	Mandibula	no	
TWB25	1121.7 LayerOS9	Bos taurus	Humerus dist	no	
TWB26	270 Layer OS3	Bos taurus	Metatarsus prox	no	
TWB27a	296 Layer OS3	Bos taurus	Mandibular tooth	no	
TWB27b	296 Layer OS3	Bos taurus	Mandibula	no	
TWB28	273 Layer OS3	Bos taurus	Phalanx I ant	no	
TWB29	354 Layer MS4	Bos taurus	Calcaneus	**yes**	*Alces alces*
TWB30a	825.2 Layer A6/7	Bos taurus	Maxilla dext, tooth	no	
TWB30b	825.2 Layer A6/7	Bos taurus	Maxilla dext, bone	no	
TWB31	1277 Layer US3	Bos taurus	Horn core sin	no	
TWB32	944-958 Layer US3	Bos taurus	Metacarpal?	**yes**	*Bison sp.*
TWB33a	1957 VII/1 Layer US3	Bos taurus	Mandibular tooth, sin	no	
TWB33b	1957 VII/1 Layer US3	Bos taurus	Mandibula sin	no	
TWB34	1957 VII/2 Layer US3	Bos taurus	Metacarpus prox	no	
TWB35	214?.5 Layer UH	Bos taurus	Metacarpus prox	no	
TWB36	1978.4 Layer UH	Bos taurus	Radius/Ulna	no	
TWB37	2005.6 Layer UH	Bos taurus	Metatarsal dist	no	
TWB38	1947.4 Layer UH	Bos taurus	M1/M2 max	no	
TWB39	2048.4 Layer MH	Bos taurus	Pelvis	no	
TWB40	2096.4 Layer MH	Bos taurus	Metatarsus prox	no	
TWB41	1848.3 Layer MH	Bos taurus	Radius dist	no	
TWB42a	1740.3 Layer MH	Bos taurus	Mandibular tooth, sin	no	
TWB42b	1740.3 Layer MH	Bos taurus	Mandibula sin	no	
TWB43	2064.1 Layer OH	Bos taurus	Metacarpus dist	no	
TWB44	**2048.1 Layer OH**	**Bos taurus**	**Metacarpus**	**yes**	***Bos primigenius***
TWB45a	2204.1/18D Layer OH	Bos taurus	Mandibular tooth, dext	no	
TWB45b	2204.1/18D Layer OH	Bos taurus	Mandibula dext	no	
TWB46	2131.1 Layer OH	Bos taurus	Metacarpus prox	no	
TWB47	2130 Layer OH	Bos taurus	Metatarsus dist	no	

3.2 Elsner J, Schibler J, Schlumbaum A (2013) Wildpferde und frühe Hauspferde in der Schweiz – zoologische und genetische Perspektiven. *Archäologie Schweiz* 36(3), p. 44-46

Abb. 1
Freiberger Pferde auf der Weide.

Wildpferde und frühe Hauspferde in der Schweiz – zoologische und genetische Perspektiven

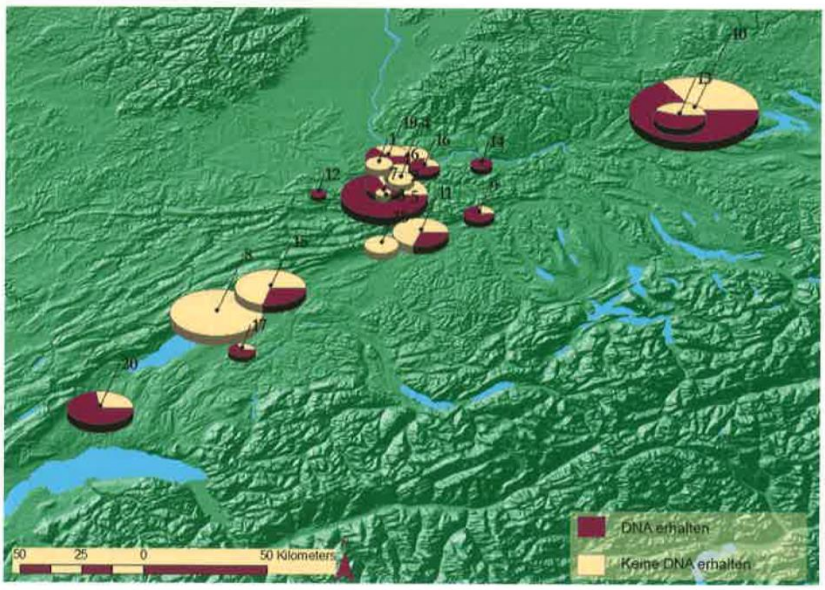
Das Freibergerpferd gilt heute als die letzte einheimische Pferderasse der Schweiz. Aber was genau bedeutet «einheimisch»? Die wilden Vorfahren unserer Hauspferde sind ausgestorben, die genetischen Mutterlinien der Hauspferde hochdivers, die Vaterlinien sehr homogen – es gibt kaum Muster, die sich bestimmten Rassen oder Regionen zuordnen lassen. Anhand von gut datierten archäologischen Equidenzähnen und -knochen haben wir die Mutterlinien (mitochondrielle DNA) von Schweizer Wild- und Hauspferden aus einem Zeitraum von über 40 000 Jahren verfolgt, Kontinuitäten und Unterbrüche dokumentiert und mit Wildpferden anderer Regionen bzw. modernen Pferden verglichen. Dieses Projekt wurde vom Schweizerischen Nationalfonds und der Freiwilligen Akademischen Gesellschaft Basel gefördert.

Die Vorfahren der Pferde entwickelten sich in Nordamerika und verbreiteten sich über die Beringstrasse über weite Teile Eurasiens und Afrikas. In ihrem Ursprungsgebiet starben die Pferde vor etwa 10 000 Jahren aus und kehrten erst im 15. Jahrhundert auf den Schiffen der spanischen Eroberer zurück.

Spätpaläolithikum

Die ältesten datierten Wildpferdfunde aus der Schweiz sind etwa 40 000 Jahre alt. Sie sind eng mit

Mutterlinien verwandt, die auch in der Folgezeit häufig in Eurasien nachgewiesen werden und unterscheiden sich genetisch deutlich von ihren nordamerikanischen Ahnen. Im Zuge der letzten Maximalvergletscherung vor 26000 bis 19000 Jahren kam es zu umfassenden Populationsverschiebungen. Der nördliche Jura blieb eisfrei und war damit ein Rückzugsort für verschiedene Spezies. Die Wildpferde aus dieser Zeit weisen genetische Muster, sog. Haplotypen, auf, die sich von denen ihrer voreiszeitlichen Vorfahren unterscheiden. Diese Muster kommen heute vor allem bei einigen Hauspferderassen in Ostasien und bei einer portugiesischen Landrasse (Sorraia) vor, wurden jedoch bisher nicht bei anderen prähistorischen Equiden entdeckt. Im Zeitraum zwischen 17000 und 12000 Jahren vor heute sind Wildpferde sehr häufig nachweisbar. Die offene Steppenlandschaft bildete einen idealen Lebensraum für Pferdeherden, die zahlreichen archäologischen Befunde zeugen von einer intensiven Pferdejagd während



2

Abb. 2
Insgesamt wurden 250 Pferdezähne und -knochen aus 22 Fundstellen untersucht, von denen 115 Proben aus 13 Fundstellen reproduzierbare mitochondrielle DNA lieferten.

Spätpaläolithische Fundstellen (ca. 41-40 000 vor heute):
1 Allschwil-Ziegelei
2 Münchenstein-Steinbruch
3 Pfeffingen-Schalberghöhle
4 Riehen-Ausserberg

Fundstellen aus dem glazialen Maximum (ca. 24-23 000 vor heute)
5 Kohlerhöhle-Untere Schicht

Magdalénienzeitliche Fundstellen (ca. 17-12 500 vor heute)
6 Birseck-Ermitage
7 Brügglihöhle
8 Champréveyres und Monruz
9 Käsloch
10 Kesslerloch
5 Kohlerhöhle-Obere Schicht
11 Rislisberghöhle
12 Roggenburg-Neumühle
13 Schweizersbild

Neolithische Fundstellen (ca. 5500-5000 vor heute)
14 Mumpf
15 Twann-Bahnhof

Spätkeltische und römische Fundstellen (ca. 0-200 n.Chr.)
16 Augusta Raurica
17 Avenches
18 Balm-Untere Fluh
19 Basel-Gasfabrik und -Münsterhügel
20 Le Mormont

Abb. 3
Vorbereitung eines Pferdezahns für die DNA-Extraktion. Mit Hilfe von standardisierten Protokollen können Verfälschungen durch moderne DNA vermieden, postmortale Schäden erkannt und die korrekte genetische Sequenz rekonstruiert werden.

des Magdalénien. Die mütterliche genetische Variabilität dieser Wildpferde ist gross, einige der damaligen Linien sind heute ausgestorben oder extrem selten. Phylogenetisch zeigen sie mehr Ähnlichkeit zu den präglazialen Equiden. Einige charakteristische Haplotypen des Magdalénien in der Region Schweizer Jura-Schwäbische Alb wurden auch in Thüringen, im Uralgebirge und in Sibirien gefunden.

Mesolithikum

Vor etwa 12 000 Jahren veränderte sich die Landschaft von einer offenen Steppe zu einem zunehmend dichter werdenden Wald. Damit einher ging wohl die Isolierung und Fragmentierung der Pferdepopulation, beträchtliche Teile wanderten ab oder starben aus. Es gibt bisher keinen archäologischen Nachweis von mesolithischen Pferden in der Schweiz.

Neolithikum

Der Mensch öffnete den dichten Wald für Ackerbau und Viehhaltung. Die vereinzelt auftauchenden Pferde waren sehr klein und kommen im Siedlungskontext zumeist dann vor, wenn die Jagdtätigkeit intensiviert war, möglicherweise ein Hinweis auf einen wilden Status. Ob es sich wirklich um Überreste früherer Populationen von Wildpferden, um neu eingewanderte oder gar vom Menschen eingeführte Hauspferde handelt, konnten wir bisher genetisch nicht feststellen, da die untersuchte Probenzahl noch zu gering ist. Neben bekannten genetischen Mustern kommen auch völlig neue Haplotypen vor.

Metallzeiten/Römerzeit

Es wird im Allgemeinen davon ausgegangen, dass die Pferde in archäologischen Befunden ab der Bronzezeit Haustiere sind. Als Domestikationsort kommt die Schweiz schon allein wegen der geringen Anzahl von Wildpferden nicht infrage. Bisher wurden noch keine bronzezeitlichen Equiden aus der Schweiz genetisch typisiert, so dass deren vermutete osteuropäische Herkunft weder bestätigt noch widerlegt werden kann. Stichprobenartig wurden genetische Untersuchungen an spätkeltischen bzw. frührömischen Pferden durchgeführt, die zumeist aus rituellem Kontext stammen. Die genetischen Muster dieser Pferde erinnern eher an moderne Hauspferde, zumal die nachgewiesenen Haplotypen bei den einheimischen paläolithischen Wildequiden nicht vorkommen. Alle modernen Hauspferderassen in der Schweiz, auch die einheimischen Freiberger, wurden einst importiert.

_Julia Elsner, Jörg Schibler, Angela Schlumbaum

Abb. 4
Archäologischer Nachweis von Hauspferden: Trensenknebel aus Hirschgeweih, osteuropäischer Typ, aus der frühen Bronzezeit. Toos-Waldi, Gem. Schönholzerswilen (TG).

Abbildungsnachweise

Schweiz. Nationalgestüt Avenches (Abb. 1)

IPNA (Abb. 3)

AA TG, D. Steiner (Abb. 4)

4. Summary of the main results and future perspectives

4.1 DNA preservation

The general aim of the thesis *Mitochondrial d-loop variation and DNA preservation in wild and domestic equids (Equus sp.) in Switzerland from the Palaeolithic to the Iron Age* was the chronological genetic investigation of archaeological horse remains from Switzerland including mitochondrial d-loop variation and the identification of coat colour and sex. The first stage comprised the evaluation of DNA preservation in equid remains from different time periods and burial conditions. Advantageously, the Pleistocene material from cave and abri (rockshelter) sites was well preserved and it was even possible to obtain genetic information from Neolithic waterlogged bones. While the positive results from the cave sites were, to some extent, expected as several studies have proven the conserving effect of those "cooling chambers" in temperate climate conditions (recently e.g. Brace *et al.*, 2012, Meyer *et al.*, 2014, Stiller *et al.*, 2014, Immel *et al.*, 2015, Lari *et al.*, 2015), it was surprising that this applied also for rock shelters which are often mere slopes (figure 6).

Figure 6: Abri site Schweizersbild, canton Schaffhausen, Switzerland, today. Picture by Bruno Sternegg, Schaffhausen-geschichte.ch.

Another fortunate outcome was the finding that DNA from waterlogged contexts is amenable to the conventional PCR approach in some cases. It was expected that water soluble DNA would largely have been washed out into the sediment, and if not, bone tissue was soaked with PCR-inhibiting humic acids which prevent amplification (Hu *et al.*, 2015). However, washing and dilution (Kemp *et al.*, 2014) of the DNA extracts permitted mtDNA and even short sequential ncDNA amplification (Schibler *et al.*, 2014) for eleven out of 54 (20 %) Neolithic specimens from Lake Biel (Twann-Bahnhof), yet not for the Magdalenian samples from Lake Neuchâtel (Monruz and Champréveyres). These lakes are in proximity on the foot of the Jura Mountains (see figure 5) with highly similar geological composition, and so the differing results might be due to the older age of the Magdalenian samples, or could also be explained by the taphonomic history of the sites. The remains left by the Magdalenian hunters who occupied the stations of Monruz and Champréveyres in spring and early summer (Leesch *et al.*, 1997, Bullinger *et al.*, 2006) were exposed to lake-water level changes (transgression, regression), including the transgression linked to the temperature rise at c. 14.7 ka BP, and thus to erosion and weathering (Coope *et al.*, 2000), to a larger degree than the settlement

remains at Twann. These contrasts in depositional environment continued until their time of excavation: the archaeological remains from Monruz and Champréveyres were recovered from beneath the lake-water level (Egloff, 1985, Egloff, 1991), whereas the Neolithic material from Twann came from groundwater-holding layers 5 m under the present settlement horizon (Orcel, 1978). The relatively superior preservation status of the Twann bones is obvious from visual inspection (figure 7).

Figure 7: Examples of bone preservation in waterlogged condition. Fragments of metatarsii from Twann (left) and Monruz (right).

The equid remains preserved in open, dry contexts also contained amplifiable DNA; the time limit here is again, according to the results from this study, the Neolithic. Palaeontological finds yielded no amplifiable DNA. Generally, the decomposition of organic material by thanatophile insects and microorganisms in temperate, open, dry environments is very effective (Albrecht *et al.*, 2003). Thus, archaeological finds from these environments are not abundant, and mostly it is only in the course of large infrastructure projects that they are detected at all.

4.1.1 DNA preservation of Swiss equid and non-equid samples from Neolithic, Iron Age and Roman dry and wetland sites

In our paper *Burial condition is the most important factor for mtDNA amplification success in Palaeolithic equid remains from the Alpine foreland* (page 13) we have closed with the proposition to investigate younger samples from different depositional contexts. Thus equid and non-equid Neolithic samples from sites with dry and waterlogged preservation, and Iron Age and Roman equid samples from dry contexts were additionally evaluated (table 1). The specimens are c. 5500 to 1700 years old. Treatment of the Neolithic samples is described in research paper two (page 36) and of the Iron Age/Roman samples in research paper three (page 83), except for 25 non-equid (*Alces*, *Bison*, *Bos*) samples from Twann and the horse bones from Augusta Raurica which were, however, handled similarly. The examination includes the potential effects of study sites (dry and wetland) and sample ages (Neolithic, Iron Age/Roman) on PCR amplification success and performance, and the amount of post mortem damage derived C→T substitutions as referred in research paper one (page 13). Iron Age and Roman samples were summarised into the same age category. Note that for some sites, the

amplification with primers Ec5f/Eac1r was successful but not reproducibly, or the success could not be repeated for longer targets, and the non-equid samples were not amplified with this primer. Thus PCR performance was estimated only for equid samples and the damage rate only for reproducible equid samples. It was not deemed reasonable to include the factors tissue type and storage time for this dataset as only four teeth stand against 74 bones, and the dates of the excavations cluster within the last 40 years, except for two samples from Basel-Gasfabrik.

Table 1: Overview of archaeological sites, ages, type of sites, and mtDNA d-loop amplification and PCR performance of positive samples (all species) expressed in percentage of total PCR repeats. Note that PCR performance does not include non-equid samples from Twann (see text).

Site	Age	Type of site	Samples with mt d-loop amplification (total samples)		PCR performance: Positive PCR (total PCR)	
			n	%	n	%
Mumpf	Neolithic	dry	2 (2)	100	5 (16)	31
Twann	Neolithic	wetland	16 (45)	36	18 (142)	13
Mormont	Iron Age	dry	14 (18)	78	38 (77)	49
Basel-Gasfabrik	Iron Age	dry	5 (6)	83	16 (52)	31
Aventicum	Iron Age/Roman	dry	3 (3)	100	3 (13)	23
Augusta Raurica	Roman	dry	3 (4)	75	14 (49)	29
		total	43 (78)	55	94 (349)	27

Results and Discussion

Depositional context has a significant impact on mt d-loop amplification success and PCR performance (here only positive samples) as specimens from dry conditions worked better in these aspects. Concerning the amount of C→T substitutions, the influence of age excelled; the older Neolithic samples were more damaged than the younger samples (figure 8, table 2).

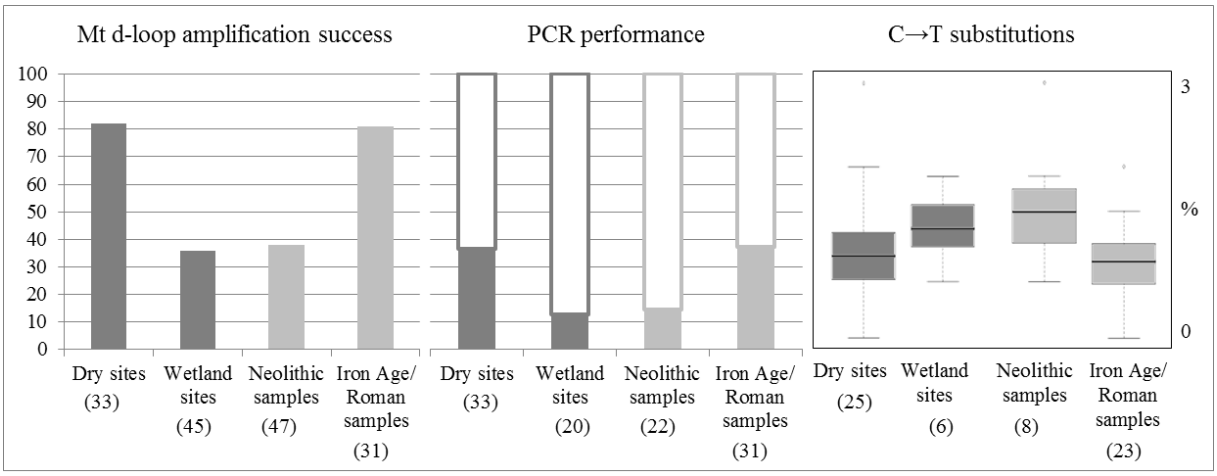


Figure 8: Influence of type of site (dry, wetland) and age (Neolithic, Iron Age/Roman) on mt d-loop amplification success: percentage of samples with amplifiable mtDNA; PCR performance: percentage of positive PCR (filled) and negative PCR attempts (hollow); and percentage (0-3 %) of C→T substitutions. Total number of samples is given in parenthesis.

Table 2: Effect of site type (wetland, dry) and age (Neolithic, Iron Age/Roman) on mt d-loop amplification, PCR performance and amount of C→T substitutions on equid samples.

	mt d-loop amplification			PCR performance		C→T substitutions	
	Df	χ^2	*p*	χ^2	*p*	F	*p*
Site	1	89.867	0.00003***	73.752	0.002**	1.96	0.2
Age	1	89.036	0.4	72.741	0.5	14.41	0.0007***

Significance code: 0.001 = ***; 0.01 = **; 0.05 = *

However, acknowledging that almost all Neolithic and none of the Iron Age and Roman samples were preserved in waterlogged conditions, the calculation was repeated comparing dry and waterlogged Neolithic samples and comparing dry sites only. Differences concerning mtDNA amplification success was detected in the Neolithic samples (table 3) and significantly less post mortem damage as represented by C→T substitutions in the Iron Age and Roman samples observed (table 4).

Table 3: Effect of site type (wetland, dry) of Neolithic samples on mt d-loop amplification, PCR performance and amount of C→T substitutions on equid samples.

	mt d-loop amplification			PCR performance		C→T substitutions	
	Df	χ^2	*p*	χ^2	*p*	F	*p*
Site type	1	58.574	0.05*	11.112	0.3	4.79	0.07

Significance code: 0.001 = ***; 0.01 = **; 0.05 = *

Table 4: Effect of age (Neolithic, Iron Age/Roman) of dry preserved samples on mt d-loop amplification, PCR performance and amount of C→T substitutions on equid samples.

	mt d-loop amplification			PCR performance		C→T substitutions	
	Df	χ^2	*p*	χ^2	*p*	F	*p*
Age	1	30.462	0.4	61.649	0.5	13.5	0.001**

Significance code: 0.001 = ***; 0.01 = **; 0.05 = *

This study shows that it is less likely to obtain mtDNA from waterlogged specimens; if they do contain amplifiable DNA, PCR performance and post mortem damage seem similar to dry preserved samples, yet note the unequal sample size (two from one dry site, 20 (with amplifiable mtDNA) or six (reproducible results for the Ec5f/Eac1r primer system) from one wetland site). In our research paper on Palaeolithic specimens wetland sites yielded no amplifiable mtDNA at all. Sample age does not influence DNA amplification and PCR performance, however, the amount of damage is significantly higher in older specimens. Yet also here, two Neolithic samples are compared to 23 Iron Age and Roman bones. It cannot be precluded that the Neolithic teeth from Mumpf were particularly affected by taphonomic processes leading to C→T substitutions. However, the general trend that older samples are less well preserved was confirmed.

Conclusion

The evaluation of factors influencing DNA preservation is continued and the dataset extended by adding equid and non-equid samples from Neolithic, Iron Age and Roman times, again from dry and waterlogged contexts. The previous result that the depositional environment is the most influential factor affecting DNA preservation is confirmed and, contrary to the Palaeolithic finds which dated from c. 47,000 to 12,000 years BP; within the short timeframe of approximately 4,000 years investigated here an increase of post mortem damage derived lesion is detectable. To add authority to this observation the pool of data has to be continuously augmented.

Within the present project, for the first time all Palaeolithic and Neolithic sites with more than a solitary remnant of a certain species have been screened for DNA preservation. The outcome of this test is very promising and applicable to other species which were abundant during those eras, for example reindeer (*Rangifer tarandus*) and red deer (*Cervus elaphus*). The additional investigation of Iron Age and Roman horse remains has shown this material is apt for genetic analyses beyond mtDNA variability. These results can be conferred to other species, for instance to explore the phenotypes of cattle and pigs.

4.2 Wild horses in Eurasia between 50,000 and 11,000 BC

The main focus of the project was the characterisation of the genetic composition and dynamics of the regional wild horse populations in the area of present-day Switzerland. The investigation of 92 archaeological remains from sites mainly adjacent to the Jura Mountains led to the conclusion that the area was not continuously populated by a panmictic deme. The lineages present during the LGM were greatly different from the preceding and succeeding lineages. The population increased after the LGM, thereby revealing a pattern diverging from the global trend. Subsequently, with progressive densification of woodland horses became rare and went locally extinct latest when human farmers demanded more and more arable land in the Neolithic.

Because the discontinuity of the Pleistocene to early Holocene populations did not meet the approach if the demographic models implemented in BEAST, each time bin was tested separately using the methods described in research paper two. Bayes Factors are presented in table 5. Only the Magdalenian dataset reached convergence after 50 million iterations for all demographic models applying both a strict and a lognomal relaxed molecular clock. The other time bins did not contain sufficient information. The Magdalenian dataset was also the only one for which the Bayes Factors gave significant support to one of the demographic models. Contrasting to the results for the diversity indices and the expansion parameters, the Constant Size model received most support, i.e. log10 over 3 (Kass *et al.*, 1995). This outcome is questioned, as the investigated partial d loop sequence probably does not contain sufficient evolutionary information for Bayesian inference, at least concerning *E. f. caballus* (see below).

Table 5: log10 Bayes Factors of wild horse sequences from Switzerland grouped in time bins. Demographic model comparisons marked with asterisk did not reach convergence for the respective dataset.

		Palaeontologic, n = 4			Badegoulian, n = 11			Magdalenian + Azilian, n = 74			Neolithic, n = 8		
		Constant Size	Skyline	GMRF Skyride	Constant Size	Skyline	GMRF Skyride	Constant Size	Skyline	GMRF Skyride	Constant Size	Skyline	GMRF Skyride
Strict molecular clock	Constant Size		-.366	*		.662	.679		1.662	3.387		.188	0.465
	Skyline	.366		*	-.662		.017	-1.662		1.725	-.188		0.277
	GMRF Skyride	*	*		-.679	-.017		-3.387	-1.725		-.465	-.277	
lognormal relaxed molecular clock	Constant Size		*	*		*	*		2.337	3.161		*	*
	Skyline	*		*	*		*	-2.337		.824	*		*
	GMRF Skyride	*	*		*	*		-3.161	-.824		*	*	

4.2.1 Wild horses from Switzerland in the context of Eurasian variation

In the following, Swiss horse sequences from before, during and after the LGM (Magdalenian and Azilian specimens) will be contextualised with Pleistocene horse data from northern Asia (Taymyr Peninsula and Sakha Republic, Russian Federation) and the Urals region (see table 11 for details) (Weinstock *et al.*, 2005, Lorenzen *et al.*, 2011, Orlando *et al.*, 2013). Figure 9 gives an overview of the localities and the haplogroup frequencies in each region.

Figure 9: Haplogroup frequencies of Pleistocene horses dating from 50,000 to 12,000 BC. Pie size corresponds to number of individuals. Samples used in table 11.

Nucleotide and haplotype diversity were estimated with the packages *pegas* (Paradis, 2010) and *seqinR* (Charif *et al.*, 2007) implemented in R (R Development Core Team, 2014) using pairwise deletion of missing data. Samples were grouped in time bins: before the LGM (BLGM, 50-27 ka BP), during the LGM (LGM, 24-20 ka BP) and after the LGM (PLGM, 17-11 ka BP), as the LGM is considered the main caesura within the development of the Eurasian environment. Climate change did affect size and configuration of the habitat and thus directly influenced horse demography.

It is apparent that horse nucleotide diversity decreases from east to west (table 6), supporting the findings of Warmuth *et al.* (2012) of an initial population expansion of wild horses in north eastern Europe. Given the small sample sizes, the results have to be treated with caution. The changing nucleotide diversity in the populations before, during and after the LGM signals different demographic developments in the regions. In northern Asia, diversity is higher during the LGM, possibly a sign of population growth, followed by a decline; whereas in the Urals region, the diversity level stays more similar. In Switzerland, significantly negative Fu's F_S results indicate population expansion after the LGM (-22.6, $p < 0.001$). Recent "global" approaches to trace horse response to climate and environmental change mainly featured Beringian (northern Asian and northern American) specimens (Lorenzen *et al.*, 2011, Orlando *et al.*, 2013) and noticed a drastic population decline after the LGM. While this is legitimate for the heartland of the so-called mammoth steppe (e. g. Sher, 1986, Guthrie, 2001), it appears that the fringe areas of this biota faced different developments. Large parts of present day Switzerland were covered by Jura and Alpine glaciers during the LGM (see figure 4) and the landscape only became accessible after the ice had retreated; then, however, it offered favourable conditions for the horse and the region might have served as a short-term retreat for the cold-adapted grazers, until, after the temperature rise around 14.7 ka BP, vegetation changed from open steppe to more and more densely forested woodland.

Table 6: Nucleotide and haplotype diversity of Eurasian Pleistocene horses based on pairwise deletion of missing nucleotides. BLGM = before the Last Glacial Maximum, LGM = Last Glacial Maximum, PLGM = after the Last Glacial Maximum.

Region	Time bin	Number of sequences	Nucleotide diversity	Haplotype diversity
Asia	BLGM	11	0.019	0.9
	LGM	3	0.022	0.67
	PLGM	2	0.019	0.5
Urals	BLGM	7	0.016	0.82
	LGM	2	0.015	0.5
	PLGM	4	0.016	0.75
Switzerland	BLGM	4	0.01	0.75
	LGM	11	0.01	0.74
	PLGM	57	0.014	0.97

As sample sizes for each region and time period are uneven, directly compared time bins were randomized (10k permutations with replacement) using nucleotide diversity to reject a statistical bias in the analyses. It transpired that most bins contained too few samples, yet the datasets Swiss LGM and Swiss PLGM (see also Research paper 2, page 36) and Swiss LGM and Asia BLGM were comparable (figure 10). Their F_{ST} values reveal great genetic differentiation (table 7A). When only directly ^{14}C dated samples from Switzerland (figure 11) are considered, we find evidence that the Swiss LGM samples are also considerable genetically distant to the Ural horses before and after LGM (table 7B). This result insistently raises the question of their origin; we are lacking data for comparison. Until now, the haplotype dominating amongst Swiss LGM horses has not been detected in other Pleistocene populations (figure 12), it is, however, frequent in some modern Eurasian breeds (see research paper two).

Table 7: F_{ST} values of Eurasian Pleistocene horses. Lower triangle: F_{ST} values, upper triangle: *p* values. Significantly comparable populations are boxed, significant values are in bold. **A** Swiss dataset without sequences with more than 40 % missing nucleotides. **B** ^{14}C dated Swiss samples.

A	Asia BLGM	Asia LGM	Asia PLGM	Ural BLGM	Ural LGM	Ural PLGM	Swiss BLGM	Swiss LGM	Swiss PLGM
Asia BLGM	-	0.3	0.6	0.5	0.6	0.5	0.7	**0.0008**	**0.03**
Asia LGM	0	-	0.4	0.4	0.8	0.3	0.4	**0.03**	0.09
Asia PLGM	0.068	0.055	-	0.3	1	0.3	0.07	**0.05**	0.1
Ural BLGM	0	0.01	0.103	-	0.4	0.6	0.7	**0.005**	0.3
Ural LGM	0	0	0	0	-	0.1	0.2	**0.01**	0.1
Ural PLGM	0	0.076	0.144	0.032	0.255	-	1	0.2	0.8
Swiss BLGM	0	0.031	0.203	0	0.203	0	-	**0.02**	0.5
Swiss LGM	**0.233**	**0.383**	**0.387**	**0.265**	**0.51**	0.162	**0.297**	-	**0.0003**
Swiss PLGM	**0.05**	0.11	0.142	0.01	0.163	0	0	**0.167**	-

B	Asia BLGM	Asia LGM	Asia PLGM	Ural BLGM	Ural LGM	Ural PLGM	Swiss BLGM	Swiss LGM	Swiss PLGM
Asia BLGM	-	0.5	0.3	0.5	0.7	0.6	0.7	**0.00006**	0.05
Asia LGM	0	-	0.4	0.3	0.8	0.3	0.4	**0.02**	0.05
Asia PLGM	0.058	0.037	-	0.3	1	0.3	0.06	**0.05**	0.1
Ural BLGM	0	0.027	0.103	-	0.4	0.6	0.8	**0.001**	0.09
Ural LGM	0	0	0	0.032	-	0.1	0.2	**0.05**	0.1
Ural PLGM	0	0.067	0.111	0	0.218	-	1	**0.01**	0.5
Swiss BLGM	0	0.038	0.203	0	0.203	0	-	**0.008**	0.4
Swiss LGM	**0.399**	**0.599**	**0.703**	**0.528**	**0.766**	**0.51**	**0.663**	-	**0**
Swiss PLGM	0.053	0.149	0.179	0.058	0.184	0	0	**0.466**	-

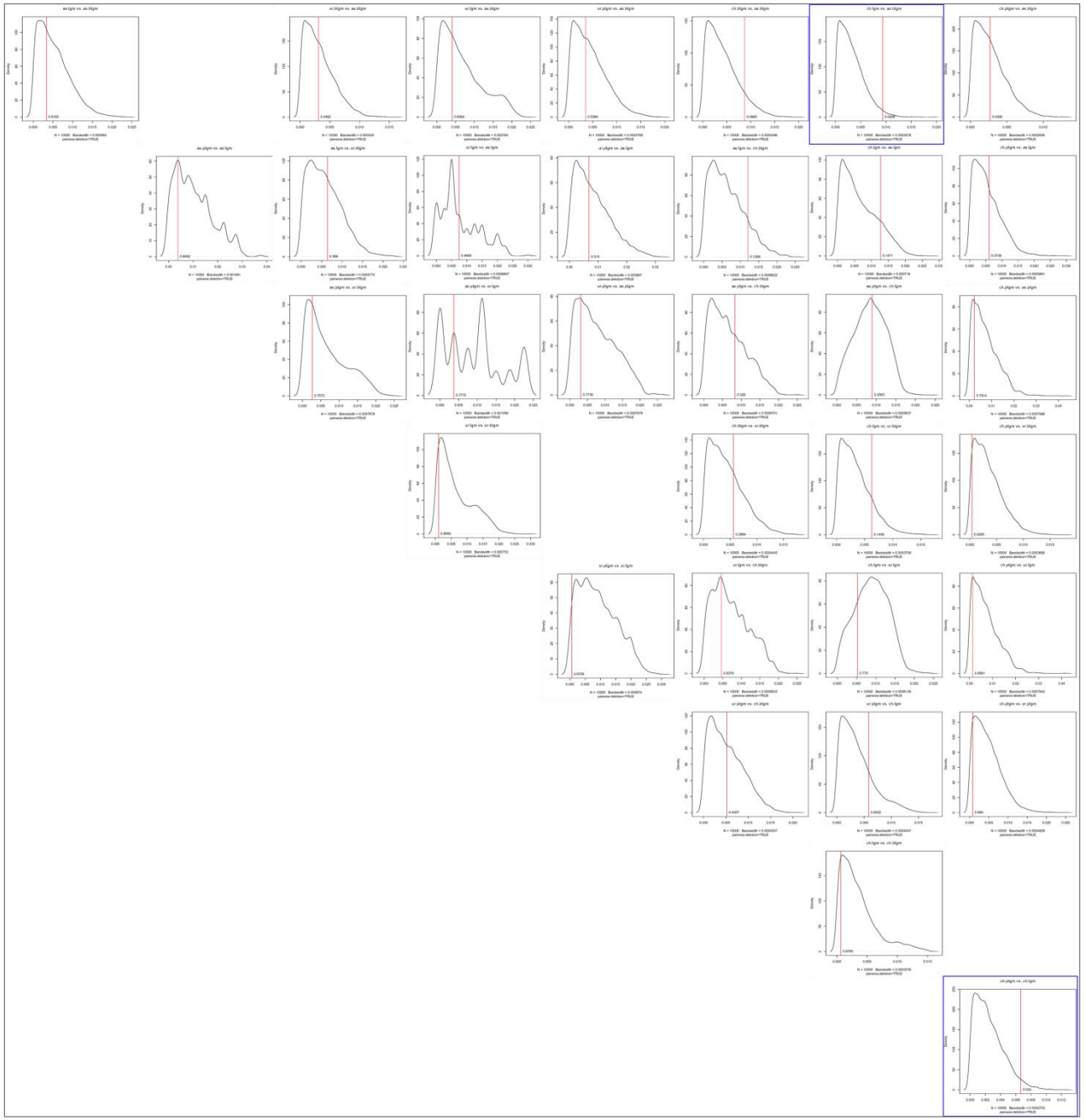


Figure 10: Density plots of randomization of Eurasian Pleistocene horse sample groups (10 k permutations with replacement). Swiss samples without sequences with more than 40 % missing nucleotides. Rejections of null hypothesis are framed blue (Swiss LGM vs. Asia BLGM 0.0208; Swiss LGM vs. Swiss PLGM 0.034).

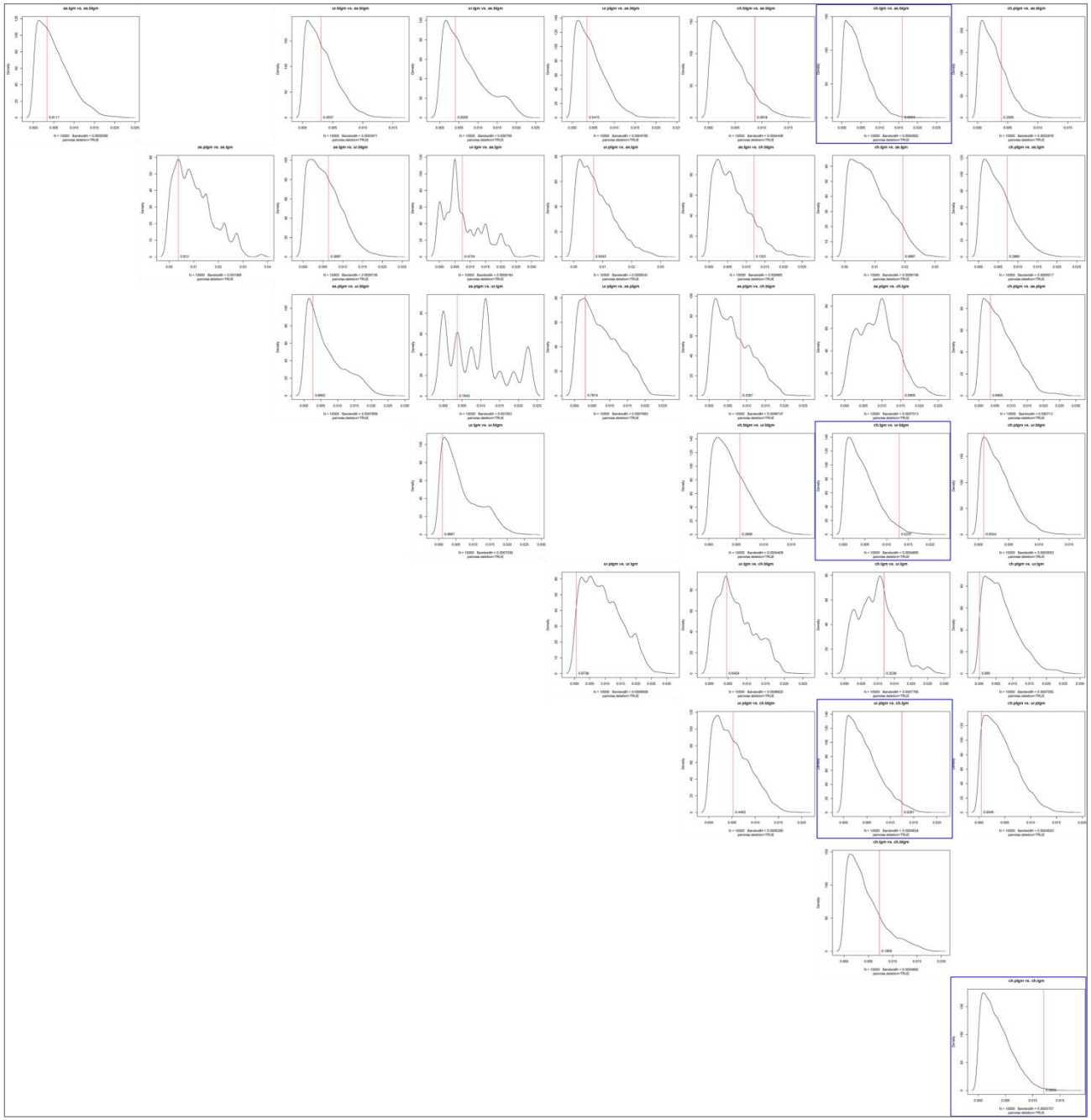


Figure 11: Density plots of randomization of Eurasian Pleistocene horse sample groups (10 k permutations with replacement). Swiss dataset with ^{14}C dated samples only. Rejections of null hypothesis are framed blue (Swiss LGM vs. Asia BLGM 0.034; Swiss LGM vs. Ural BLGM 0.0237; Swiss LGM vs. Ural PLGM 0.0267; Swiss LGM vs. Swiss PLGM 0.0055).

Diversity indices and F_{ST} values have indicated that the LGM was interrupting the demographic continuity of Eurasian horse populations, although not in a linear way. Especially the Swiss lineages were genetically different from horses preceding and succeeding in all regions investigated. Figure 12 illustrates this result.

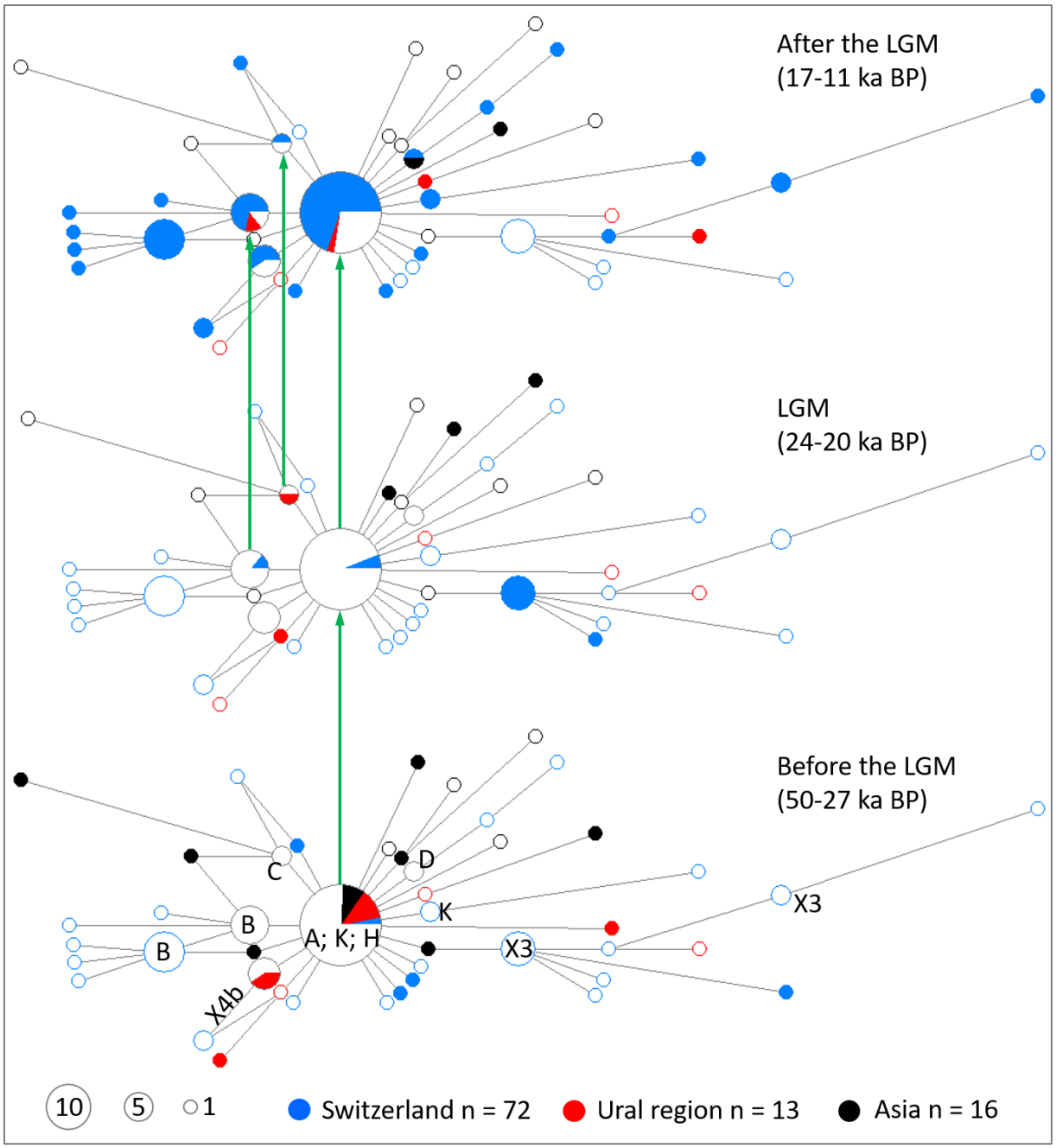


Figure 12: Median Joining Network of Eurasian Pleistocene horse sequences. Nodes proportional to number of sequences, length of branches to number of mutations. Hollow nodes represent haplotypes not present in respective time period. Green arrows indicate continuing haplotypes. Nomenclature follows Cieslak *et al.* (2010). Weighting parameters are given in table 8.

Table 8: Parameters for weighting of nucleotide positions for Median Joining Network analysis based on Eurasian Pleistocene horse mtDNA d-loop sequences.

Positions in reference sequence NC_001640	15494	15496	15511	15519	15521	15526	15533	15534	15536	15540	15542	15544	15550	15551	15558	15565	15573	15581	15583	15584	15585	15587	15595	15597	15598	15600	15602	
Nucleotide sequence of majority	T	A	G	C	G	T	A	C	T	A	C	T	C	C	G	C	T	G	A	C	A	C	A	A	T	G	T	
Number of changes to A			1		1										1			1								1		
Number of changes to T				1				1			13		3	1		1				1		1						
Number of changes to C	1					2			6			6					1							1		4	
Number of changes to G		1					1			8									1		55		1	7				
Number of transitions	1	1	1	1	1	2	1	1	6	8	13	6	3	1	1	1	1	1	1	1	55	1	1	7	1	1	4	
Number of transversions																												
In Swiss dataset (78)			1	1				1	6	6	12	4	2	1			1	1	1	1	41	1		3			3	
In Ural dataset (13)					1	1				2	1	1	1			1					8			1		1		
In Asian dataset (16)	1	1				1	1					1			1						6		1	3	1		1	
Total	1	1	1	1	1	2	1	1	6	8	13	6	3	1	1	1	1	1	1	1	55	1	1	7	1	1	4	
Calculated weight	49	49	49	49	49	48	49	49	44	42	37	44	47	49	49	49	49	49	49	49	0	49	49	43	49	49	46	
Actual weight in MJN	**49**	**49**	**49**	**49**	**49**	**48**	**49**	**49**	**44**	**42**	**50**	**44**	**47**	**49**	**49**	**49**	**49**	**49**	**49**	**49**	**0**	**49**	**49**	**0**	**49**	**49**	**46**	

Positions in reference sequence NC_001640	15603	15604	15610	15611	15612	15617	15623	15635	15642	15646	15649	15650	15651	15654	15659	15666	15667	15672	15683	15703	15712	15718	15720	15726	15740	15747	15753	
Nucleotide sequence of majority	T	G	T	G	A	T	T	C	C	C	A	A	G	C	T	G	A	G	C	T	A	C	A	G	A	A	G	total
Number of changes to A		16		1					1				1			9		1						1			1	36
Number of changes to T								3	1	1				1					1			5						34
Number of changes to C	1		1			20	1								11					17							72
Number of changes to G					1						5	39					1				1		2		3	4		130
Number of transitions	1	16	1	1	1	20	1	3	1	1	5	39	1	1	11	9	1	1	1	17	1	5	2	1	3	4	1	271
Number of transversions									1																			1
In Swiss dataset (78)		10	1	1		18	1	3	2	1	2	28		1	10	7				8	1	2	2		3		1	188
In Ural dataset (13)		2			1	1					1	4				1			1	4		2						35
In Asian dataset (16)	1	4				1					2	7	1		1	1	1	1		5		1		1		4		49
Total	1	16	1	1	1	20	1	3	2	1	5	39	1	1	11	9	1	1	1	17	1	5	2	1	3	4	1	272
Calculated weight	49	34	49	49	49	30	49	47	48	49	45	11	49	49	39	41	49	0	0	33	49	45	48	49	47	46	49	
Actual weight in MJN	**49**	**0**	**49**	**49**	**49**	**50**	**49**	**47**	**48**	**49**	**45**	**0**	**49**	**49**	**50**	**50**	**49**	**0**	**0**	**0**	**49**	**45**	**48**	**49**	**47**	**46**	**49**	

To explore the demographic development of the Eurasian horse populations we used the Bayesian phylogenetic inference package BEAST 1.8.0 (Drummond *et al.*, 2012). ModelGenerator v0.85 (Keane *et al.*, 2006) suggested the TN93+Γ (Tamura *et al.*, 1993) model of nucleotide substitution (AIC and BIC). The evolutionary rate was calibrated using the age of the specimens. We tested Constant Size (Lemey *et al.*, 2010), Bayesian Skyline (Drummond *et al.*, 2005) and Gaussian Markov Random Field (GMRF) Skyride (Minin *et al.*, 2008) demographic models applying both a strict and an uncorrelated lognormal relaxed molecular clock (ucld, initial value $1e^{-7}$, upper $2e^{-6}$, lower $1e^{-8}$) (Drummond *et al.*, 2006). Markov Chain Monte Carlo (MCMC) were run for 500 million iterations with samples drawn from the posterior every 50 k iterations. Empty alignments were created to verify the outcome resulted from the data and not from the prior information. Convergence and mixing were evaluated using Tracer 1.5 (Rambaut *et al.*, 2007). The first 10 % of runs were discarded as burn-in. To choose the appropriate model Bayes Factors were collected from Tracer 1.5. It transpired only the constant size demographic models received enough support (table 9), i.e. log10 over 3 (Kass *et al.*, 1995), which contradicts the observations based on diversity indices.

Table 9: log10 Bayes Factors for Eurasian Pleistocene horse sequences applying the constant, skyline, and Skyride demographic models with both strict and relaxed molecular clock.

log10 Bayes Factors	constant strict	skyline strict	skyride strict	ln10 Bayes Factors	constant relaxed	skyline relaxed	skyride relaxed
constant strict		0.961	2.301	constant relaxed		1.484	2.953
skyline strict	-0.961		1.339	skyline relaxed	-1.484		1.469
skyride strict	-2.301	-1.339		skyride relaxed	-2.953	-1.469	

This might be explained by i) the assumption of a continuous population by the approach implemented in BEAST is not met, and ii) the partial d-loop does not contain enough evolutionary information. Considering the result of the comparison of Bayesian skyline plots of whole mitogenomes and 241 bp of mt d-loop (investigated target in ancient sequences) of the same set of sequences of modern horses (figure 13), the second explanation seems likely. In both the datasets from Lippold *et al.* (2011b) and Achilli *et al.* (2012), the expansion signal (figures 13A and 13C) is not caught when the dataset is pruned, and the margin of the standard error is considerably widened (figures 13B and 13D). As the analysis of partial d-loop sequences with Bayesian inference methods have yielded reasonable results for other species (Campos *et al.*, 2010b, Stiller *et al.*, 2010, Welch *et al.*, 2012), it is possible the extensive heteroplasmy of the control region of the horse dilutes the analysis concerning *E. f. caballus* (Xu *et al.*, 1994).

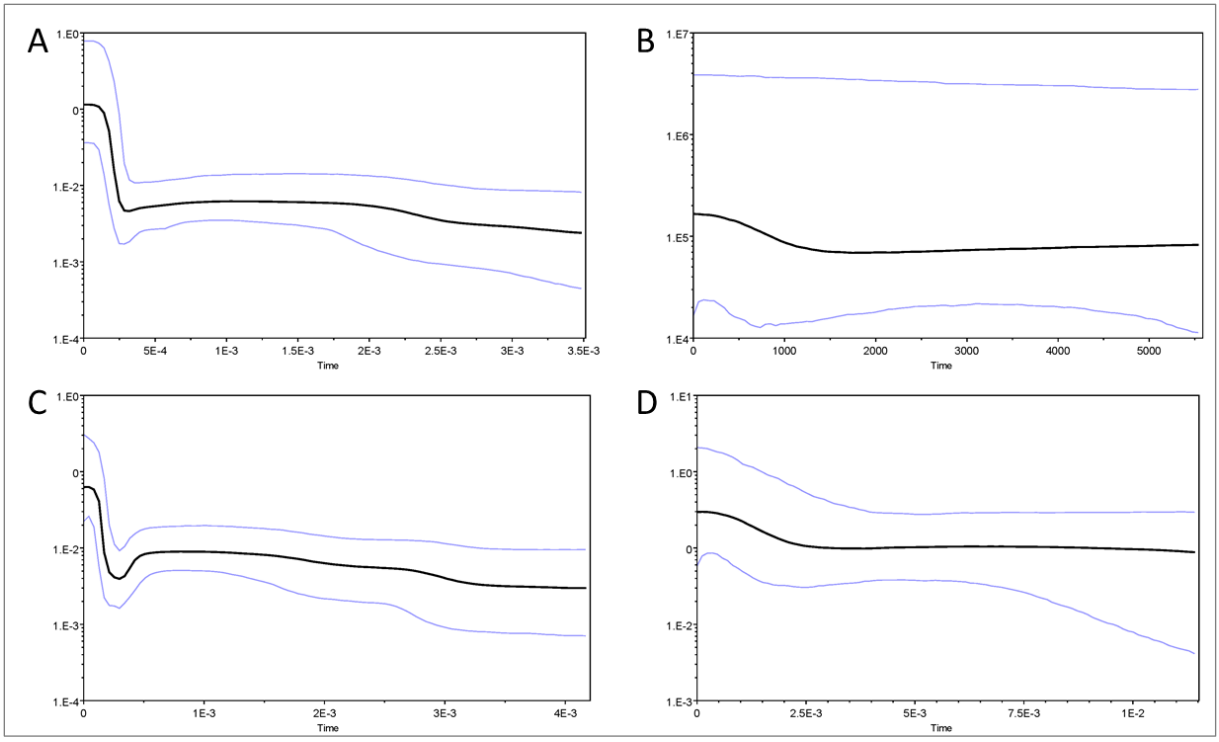


Figure 13: Bayesian Skyline plots of modern horse sequences. **A** Complete mitochondrial sequences of 63 horses (Lippold *et al.*, 2011b); **B** Same set of sequences pruned to partial d-loop section (241 bp) which was amplified from ancient samples; **C** Complete mitochondrial sequences of 83 horses (Achilli *et al.*, 2012); **D** Same set of sequences pruned to partial d-loop section (241 bp)which was amplified from ancient samples.

In conclusion, the summary of Eurasian Pleistocene horse sequences in order to draw a picture of general demographic trajectory leads to simplification at best. The divergent environment in the mammoth steppe and its fringe areas caused different developments. To meet the approach of a Eurasian picture, it is necessary to pursue a comprehensive geographically and chronologically dense sampling pattern, comparably to the Swiss example that comprised all known Pleistocene horse specimens.

4.3 Domestic horses in Eurasia between 2,000 BC and 1,000 AD

Finally, Iron Age and Roman domestic horses were investigated. The specific questions addressed concerned the characterisation of the matrilinear diversity and coat colouration of Iron Age horses and whether they differed genetically from the rare but consistently occurring large horses (withers height > 140 cm) which appear in Celtic contexts. The archaeozoologically defined standard Celtic horse was very small (110-130 cm). It transpired that while the female lineages were highly diverse, coat colourations were limited to the basic colours bay, black and chestnut. The few larger horses in our dataset were not genetically distinct from the smaller majority. Breeding interests were apparently focussed on good performance and low maintenance horses, and to avoid inbreeding. This emphasises the skill and expertise Celtic stock keepers executed on this highly valuable and prestigious animal.

It seems that when encountering horse remains in Celtic archaeological contexts, there is often an association with wealth and prestige. Along with decorated weaponry, prestigious tableware, and luxurious jewellery, decorated chariots and horse harness are the characteristics of Celtic elite (figure 14) (e.g. Reynolds, 1995, 191). Horses were used to pull chariots to the battlefield (bell. gall. 5, 19: Möller, 2013), Celtic warriors rode to battle and jumped off horseback to fight (bell. gall. 4, 2: Möller, 2013), possibly they also fought on horseback (Marcellus, 6: Perrin, 1917, 449). The deployed war-horse was supposedly a stallion (Nobis, 1973, Lauwerier *et al.*, 1992, Kunst, 2000). In the Celtic ritual context, however, there seems to be a differentiation between animals whose cadavers were exhibited – mostly older males, and those which were eaten in ritual meals – mostly younger and both sexes (Méniel, 1997). However, the encountered horse remains generally show a greater variability indicating different selection criteria, possibly similar to human (warrior) sacrifices (Brunaux, 1997, Méniel, 1997).

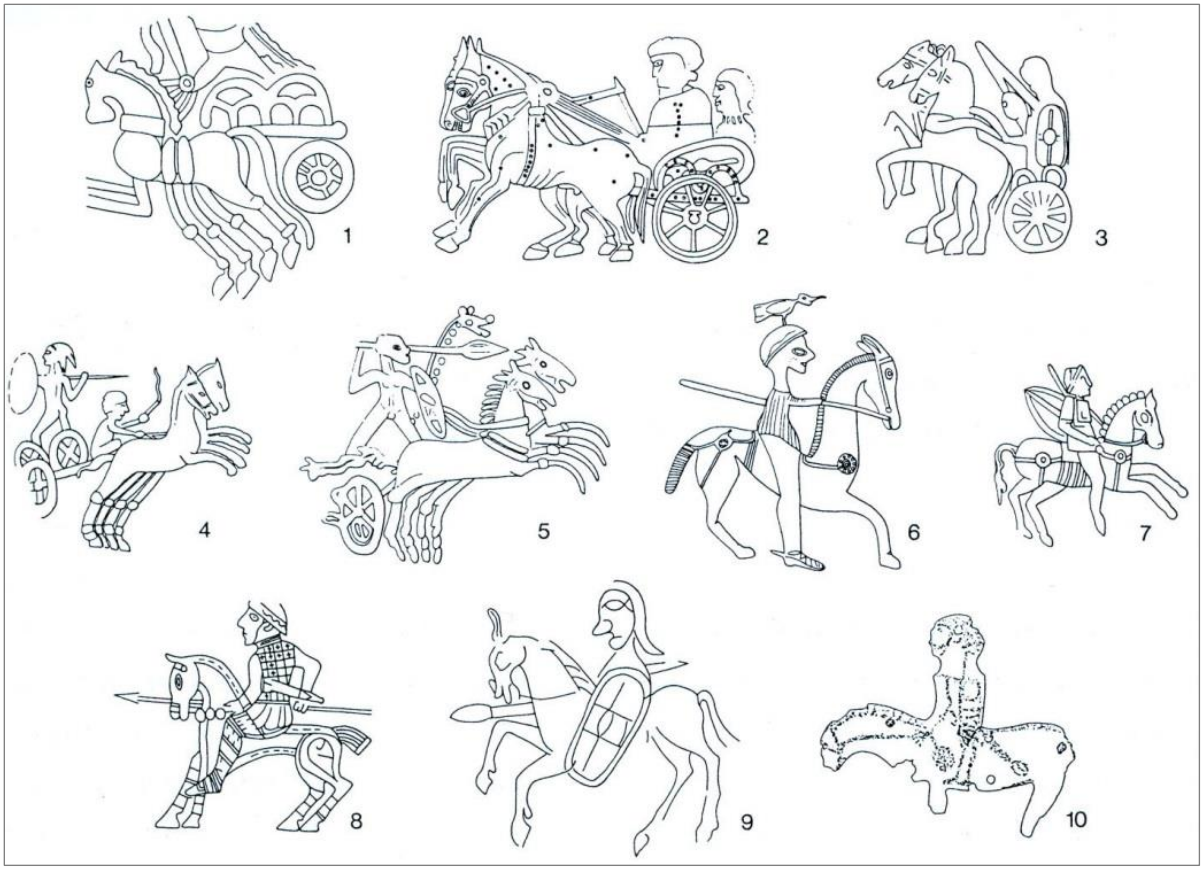


Figure 14: Representation of Celtic riders and harnessed horses from different objects. 1 – Gaulish coin, 60 BC; 2, 3 – sepulchral steles from Padua, 3rd century BC; 4 – Roman coin, 50 BC; 5 – Roman coin, 118/92 BC; 6 – silver cauldron from Gundestrup, 2nd-1st century BC; 7 – Britannic coin, 1-25 AD; 8 – scabbard from Hallstatt, 400-350 BC; 9 – rider with typical Celtic oval buckler, lance, helmet with crest on a bronze votive plate from Este near Padua, 3rd century BC; 10 – rider with sword and head trophy on bronze mounting from a carriage burial in Kärlich, 5th century BC. Taken from Rieckhoff *et al.* (2001, 145).

4.3.1 The Horses from Mormont – archaeozoological framework

The deposition of complete carcasses in Mormont provides a rare case where information on age, sex and withers height is obtainable. The mares had considerably higher ages at death (> 10 years, n = 11) than the stallions (5.5 years, n = 18) (Méniel, 2014, 19). This suggests different usage of the sexes, with mares kept for breeding and stallions for performance, e.g. military purposes, likely exhausting them at an early age. The investigation of horse burials from two Roman auxiliary forts in Lower Austria (Kunst, 2000) and The Netherlands (Lauwerier *et al.*, 1992) showed that most of them died between the ages of five to nine years. Apparently the main deployment age for stallions in the military was four to five years, as suggested by the examination of 30 horses, probably killed in a battle during the Batavian revolt in AD 69, from Krefeld-Gellep in the Lower Rhine area, Germany (Nobis, 1973). Arguing against a pure military context in Mormont is, apart from the striking absence of weaponry from the depositions, the more or less balanced sex ratio (22 females and 34 males were identifiable) and the presence of three foals (≤ 1 year). The presence of a herd-like composition would be interpreted as a breeding stock of a stud farm. In light of the suggested reading of the site as a temporary camp of refugees or migrants (Méniel, 2014, 200f) the question why the horses were killed off then on the hill of Mormont remains open, and the

ritual interpretation of the activities come to the fore despite obvious differences to the archetypical Celtic sanctuary sites like La Tène, Gournay-sur-Aronde or Ribemont (Méniel, 2014, 195).

The mares measured between 115 cm and 127.5 cm and the stallions 118-127 cm and 141.5-148.5 cm. Generally, if raised under similar conditions mature horses do not display sexual dimorphism though there might be differences in bone robusticity and skeletal proportions (Johnstone, 2004, 111f). The two larger individuals thus received a different treatment, for instance high protein fodder in their early years that enabled them to reach their full genetic growth potential. Stable isotope analysis has indeed shown that the largest horse had received a supplement of C_4 plants, in this case millet, (~ 30 %) at least during his first four years (Nuviala *et al.*, 2014). However, millet was not part of the usual horse diet in Roman studs, which consisted of grass, wheat, barley, and legumes (Peters, 1998, 142, Johnstone, 2004, 45). To feed the animal on millet might have been an attempt by Celtic stock breeders to replicate a big horse.

4.4 Perspectives on the elusive domestication process of horses

The diversity of matrilineages amongst Celtic horses is striking. This outcome is contextualised with wild and domestic horse sequences from the literature and from research paper two (figure 15). The nucleotide diversity of the domestic horses is higher (0.03±0.02) than nucleotide diversity of Eurasian wild horses (0.02±0.01), and that is matched by the Swiss Iron Age horses alone (0.03±0.02). This is striking because the diversity of a wild population sampled from north-eastern Asia to south-western Europe over a time period of c. 40 ka is expected to be higher than the diversity of a domestic population that covers about the same spatial range but only 4 ka, and the more so when the domestic samples stem from only one locality and a time frame of only about two centuries. A domestic population has had to pass through two bottlenecks, the first one being selection for domestication itself, and the second one being selection for dispersal. More ancestral variation and higher diversity persist in the centre of domestication origin; this is being pruned through successive colonisation events out of the centre, as exemplified by cattle (*Bos taurus*) (Troy *et al.*, 2001). It is hypothesised that horses had a similar or even lower number of founding female lineages at the beginning of domestication compared to cattle (Jansen *et al.*, 2002, Lippold *et al.*, 2011b, Bollongino *et al.*, 2012). The estimations for horses are, however, based on the number of lineages in the respective investigated modern horse samples and not on serial coalescent simulation and approximate Bayesian computation as for cattle, and thus most probably much too low. Complicating the comparability of the founding numbers of cows and mares is the different domestication development which involved repeated restocking with wild mares (Der Sarkissian *et al.* (2015) suggest this had been practiced until very recent times) and the question whether these are to be considered founders as well. Bollongino *et al.* (2012) state that the low number of 80 founding cows was consistent with the restricted area the domestication process originated in, and also with the difficulties of sustained management and breeding. Contrastingly, most authors agree that horse domestication involved a large sample of wild horse matrilineages, supposedly from different regions in Eurasia (e.g. Lippold *et al.*, 2011b, Achilli *et al.*, 2012), some even propose that there was no bottleneck situation at all (Gerbault *et al.*, 2012). Moreover, after the initial steps of the domestication process had been accomplished – probably applying cattle management techniques (Anthony, 2007, 200), the acquisition of additional wild mares might have been facilitated using a domestic stallion to gather and guard the harem group. Considering this, the number of founding mares is expected to be higher than the number of founding cows.

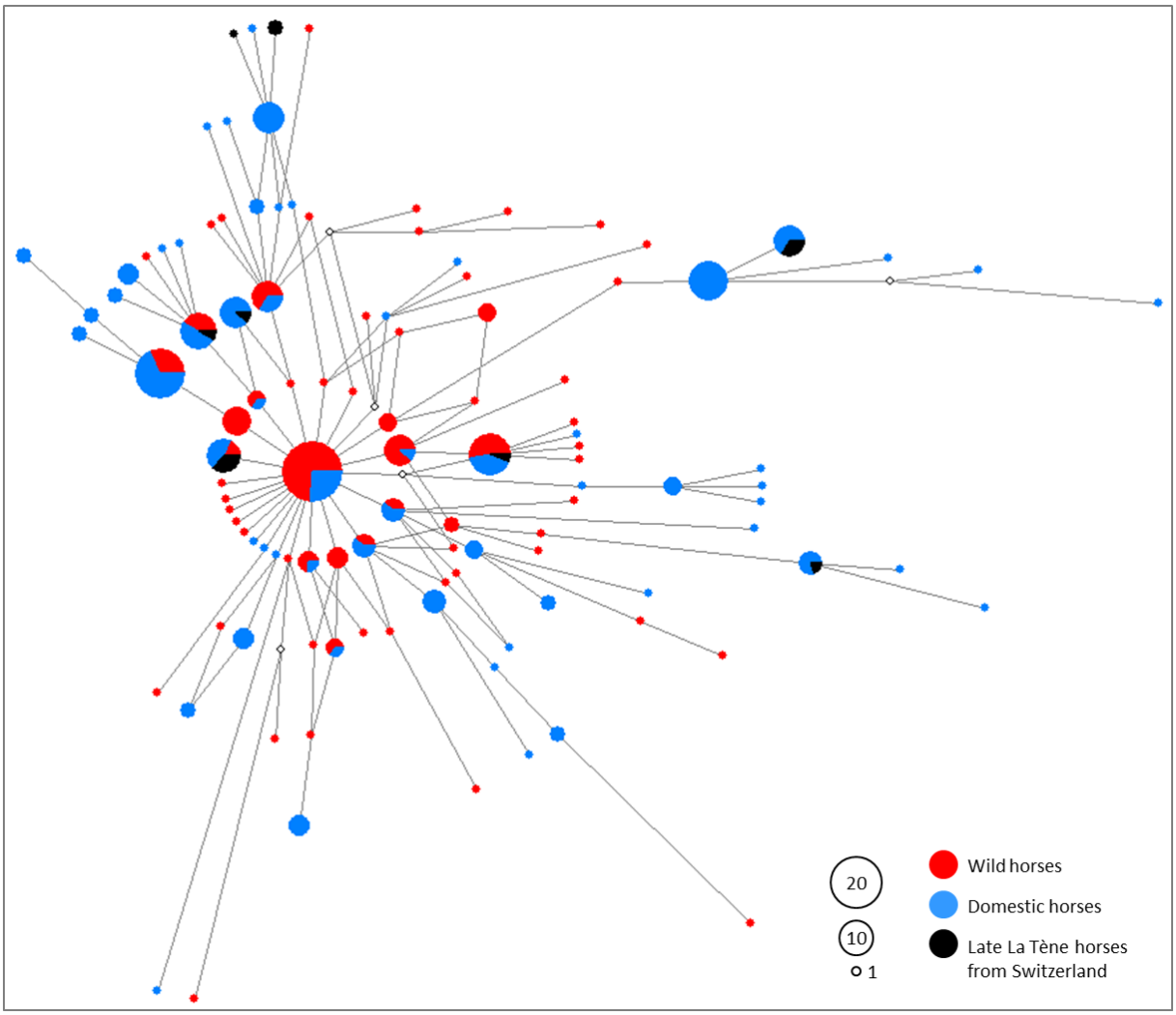


Figure 15: Median Joining network of 130 wild and 186 domestic horses including 14 Late La Tène horses from Switzerland. The weighting parameters for network construction are found in table 10; the individual sequences used in table 11.

The diversity of modern horses today is significantly higher in breeds from Iberia and the Caspian Sea region (Warmuth *et al.*, 2011). Particularly the region north of the Black- and Caspian Sea is nominated by evidence from various disciplines as a domestication hotspot for horses (Anthony, 2007, Ludwig *et al.*, 2009, Outram *et al.*, 2009, Warmuth *et al.*, 2012). It is certain that the bearers of the Yamnaya cultural horizon (3,600-2,300 BC), who massively spread from the Pontic-Caspian-Ural steppes into Central Europe around 2,500 BC (Haak *et al.*, 2015), brought the domestic horse with them – the speed of their spread would not have been possible without horsepower. They were possibly primarily responsible for the rapid dispersal of the animal in this region as the horse did not only come as an additional source of meat, but also as mount and pulling wheeled vehicles which had only recently been invented (Anthony, 2007, 65). Unfortunately, genetic information from horse remains from the Yamnaya horizon has not been published so far, and nor has data from the iconic archaeological sites Dereivka (Sredni Stog horizon, preceding Yamnaya) and Botai (Botai horizon, approximately contemporaneous to Yamnaya). Both sites contained high frequencies of horse bones and have been standing in the centre of debate as domestication hotspots for decades (see Anthony, 2007, 213ff for a summary).

The haplogroup frequencies from domestic horses dating between 2,500 BC and 1,000 AD show high diversity in different regions (figure 16). On the one hand, East Asia (China, Korea) and Europe (excluding Iberia) contain almost consistent patterns of haplogroup variety each, thinning out towards the fringes. On the other hand, the compositions in the central Asian sites (Altai, Novosibirsk and Siberia) are very different from each other. Some have more similarities to East Asia (Novosibirsk sites), some to Europe (Altai sites). This could be due to multiple domestication localities, but also to the development of domestic horse dispersal, i.e. the migrations, raids or trade networks of equestrianised peoples. Iberia is obviously a special case as a unique haplogroup (H1) characterised by a one base pair deletion, dominated in the region. This signifies that local mares were domesticated. The concurrent presence of European haplogroups poses the question of whether domestication was autochthonous or triggered by external influences. However, a reduced variation of Iberian and European matrilineages persisted in the wild population as well (Cieslak *et al.*, 2010).

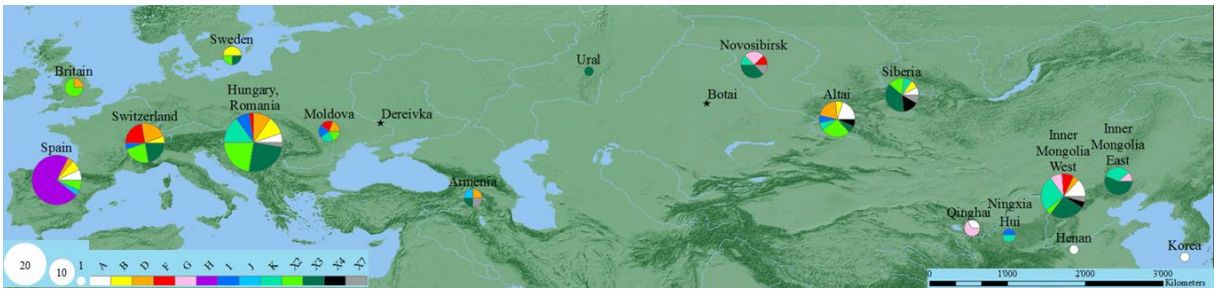


Figure 16: Haplogroup frequencies from domestic horses dating from 2,500 BC to 1,000 AD. Pie size corresponds to number of individuals. Samples used in table 11.

In conclusion, in order to fill the gaps on the map and in our knowledge, more data has to be obtained, particularly from the large horse bone assemblages from Dereivka and Botai. The advanced progress that has been achieved in the recent years concerning both sequencing technique and understanding the genetics of Pleistocene horses and underlying domestication (Schubert *et al.*, 2014, Imsland *et al.*, 2016) can also provide valuable contributions to the debate about the wild or domestic status of the horses from these sites. With key sites like those and others investigated, it may be possible in the future to reconstruct the dispersal of the domestic horse from the original centres to regions all over the world, including Switzerland.

Table 10: Parameters for weighting of nucleotide positions for Median Joining Network analysis based on 130 wild and 186 domestic horse mtDNA d-loop sequences including 14 Late La Tène horses from Switzerland.

Positions in reference sequence NC_001640	15494	15495	15496	15507	15509	15510	15511	15519	15521	15526	15528	15532	-	15533	15534	15536	15538	15539	15540	15541	15542	15544	15546	15550	15551	15558	15565	15567	15573	15581	15583	15584	15585	15587	15595	15596	15597	15598	15600
Nucleotide sequence of majority	T	C	A	T	C	T	G	C	G	T	C	C	-	A	C	T	A	C	A	C	C	T	C	C	C	G	C	T	T	G	A	C	G	C	A	A	A	T	G
Number of changes to A				1		1	1		8																	1				1			134						4
Number of changes to T		5			1			1			1				28			1		1	37		1	3	1		1					1		1				
Number of changes to C	28									10						35						9						1				1					8	
Number of changes to G			28														7		15									1			2				3	4	48		
Insertions/Deletions												x	x	x																								
Number of transitions	28	5	28		1		1	1	8	10	1				28	35	7	1	15	1	37	9	1	3	1	1	1		1	1	2	1	134	1	3	4	48	8	4
Number of transversions				1		1																						1					1						
Total	28	5	28	1	1	1	1	1	8	10	1				28	35	7	1	15	1	37	9	1	3	1	1	1	1	1	1	2	1	135	1	3	4	48	8	4
Weight	**22**	**45**	**22**	**49**	**49**	**49**	**49**	**49**	**42**	**40**	**49**	**99**	**99**	**99**	**22**	**15**	**43**	**49**	**35**	**49**	**13**	**41**	**49**	**47**	**49**	**49**	**49**	**49**	**49**	**49**	**48**	**49**	**0**	**49**	**47**	**46**	**2**	**42**	**46**

Positions in reference sequence NC_001640	15601	15602	15603	15604	15610	15611	15612	15615	15616	15617	15623	15626	15631	15632	15635	15638	15642	15646	15647	15649	15650	15651	15654	15657	15659	15660	15666	15667	15697	15703	15709	15718	15720	15723	15726	15737	15740	
Nucleotide sequence of majority	T	T	T	G	T	G	A	A	A	T	T	A	C	T	C	T	C	C	A	A	A	G	A	T	T	A	G	A	T	T	C	C	A	C	G	C	G	Total
Number of changes to A				68		1											1					2					41								8				272
Number of changes to T															31		1	1					1								9	17		2					145
Number of changes to C	11	44	27		1					40	2			1		1								1	30				2	65					7			324
Number of changes to G							1	8	6			2	1						2	39	94					1		10				23				19		314
Insertions/Deletions																																						
Number of transitions	11	44	27	68	1	1	1	8	6	40	2	2		1	31	1	1	1	2	39	94	2		1	30	1	41	10	2	65	9	17	23	2	8	7	19	1048
Number of transversions													1				1						1															7
Total	11	44	27	68	1	1	1	8	6	40	2	2	1	1	31	1	2	1	2	39	94	2	1	1	30	1	41	10	2	65	9	17	23	2	8	7	19	1055
Weight	**39**	**6**	**23**	**0**	**49**	**49**	**49**	**42**	**44**	**10**	**48**	**48**	**49**	**49**	**19**	**49**	**48**	**49**	**48**	**11**	**0**	**48**	**49**	**49**	**20**	**49**	**9**	**40**	**48**	**0**	**41**	**33**	**27**	**48**	**42**	**43**	**31**	

Table 11: Sequences used for Median Joining Network analysis. [a] sequences are part of draft full genomes which are obtainable as SRA-Illumina runs on GenBank. [b] sequences are not published on GenBank. [c] sequences are only published in Genbank.

Origin	Date	GenBank accession code	Reference
Wild horses			
Germany (south)	50-14,000 BP	DQ007558/DQ007611; DQ007556/DQ007609; DQ007591; DQ007590	Weinstock *et al.* (2005)
		FJ204352	Cieslak *et al.* (2010)
Germany (east)	16,500 BP	FJ204347; FJ204348; FJ204351	Cieslak *et al.* (2010)
Ireland	32,000 BP	DQ327850	McGahern *et al.* (2006b)
Moldova	6,3-5,100 BP	FJ204358	Cieslak *et al.* (2010)
Romania	6,3-5,100 BP	FJ204355; FJ204356	Cieslak *et al.* (2010)
Russian Federation (Taymyr peninsula)	43-16,000 BP	upon request [a]	Orlando *et al.* (2013)
Russian Federation (Novosibirsk islands, Sakha Republic)	40-20,000 BP	upon request [a]	Orlando *et al.* (2013)
		JN570964-JN570966	Lorenzen *et al.* (2011)
		FJ204314-FJ204318	Cieslak *et al.* (2010)
Russian Federation (Lena river delta, Sakha Republic)	36-31,000 BP	JN570958; JN570959	Lorenzen *et al.* (2011)
		DQ007577	Weinstock *et al.* (2005)
Russian Federation (Kolyma lowlands, Sakha Republic)	38-17,000 BP	JN570957; JN570961	Lorenzen *et al.* (2011)
Russian Federation (Yana river basin, Sakha Republic)	22,500 BP	JN570960	Lorenzen *et al.* (2011)
Russian Federation (Urals)	46-17,000 BP	JN570954; JN570972-JN570982; JN570993	Lorenzen *et al.* (2011)
Spain	7,5-6,800 BP	FJ204380-FJ204386; FJ204390; HM802276; HM802280	Cieslak *et al.* (2010)
Switzerland	41-5,000 BP	KC893753-KC893758; KC893763; KC893764; KC893766; KC893769; KC893771; KC893772; KC893774-KC893778; KC893781; KC893783-KC893785; KC893788-KC893790; KC893792; KC893793; KC893795; KC893797; KC893798; KC893800-KC893827; KC893829-KC893844	Elsner *et al.* (2017)
Turkey	5,850 BP	FJ204349	Cieslak *et al.* (2010)
Ukraine	11-5,100 BP	FJ204354; FJ204359-FJ204364	Cieslak *et al.* (2010)
Domestic horses			
Armenia	2,000-800 BC	FJ204350; FJ204369-FJ204371	Cieslak *et al.* (2010)

British Isles	190-700 AD	DQ327848; DQ327851 upon request[b]	McGahern *et al.* (2006b) Bower *et al.* (2013)
China	2,000-500 BC	FJ204377-FJ204379 EU931584-EU931609 DQ900922-DQ900930	Cieslak *et al.* (2010) Cai *et al.* (2009) Cai *et al.* (2007)
Hungary	500-900 AD	FJ624150-FJ624157; GQ119628-GQ119631; EU093030-EU093044; EU559575-EU559585	Priskin *et al.* (2010)
Kazakhstan (Altai)	300 BC	AJ876883-AJ876892	Keyser-Tracqui *et al.* (2005)
Korea	750 AD	AY049720	Jung *et al.* (2002)
Moldova	1,5-1,000 BC	FJ204372-FJ204376	Cieslak *et al.* (2010)
Mongolia (Altai)	400-300 BC	FJ204344-FJ204346	Cieslak *et al.* (2010)
Romania	1,5-1,000 BC	FJ204366; FJ204367	Cieslak *et al.* (2010)
Russian Federation (Altai)	900-300 BC	FJ204319; FJ204329	Cieslak *et al.* (2010)
Russian Federation (Novosibirsk)	2,000 BC	FJ204320-FJ204328	Cieslak *et al.* (2010)
Russian Federation (Siberia)	600 BC	FJ204330-FJ204343	Cieslak *et al.* (2010)
Russian Federation (Urals)	213 BC	DQ007571	Weinstock *et al.* (2005)
Spain	2,500 BC-1,000 AD	DQ683525-DQ683544	Lira *et al.* (2010)
Sweden	200-500 AD	FJ204387-FJ204389; FJ204391; FJ204392; HM802277-HM802279; HM802281 AF326676-AF326679	Cieslak *et al.* (2010) Vila *et al.* (2001)
Switzerland	100 BC-700 AD	KC893845-KC893847[c]; KC893849; KC893850; KC893852-KC893860; KC893862; KC893864-KC893866	Elsner *et al.* (2016)

5. Conclusion

The general aim of the thesis was a chronological genetic investigation of archaeological horse remains from Switzerland including mitochondrial d-loop variation, coat colour and sex identification. Three main subjects were in the focus.

Firstly, mtDNA preservation of archaeologic horse remains in the context of different burial conditions. A systematic synthesis of the influence of different burial conditions on DNA amplification success concerning teeth and bones from open dry and wetland sites and caves from the Pleistocene until Roman times has been made. It led to the conclusion that Pleistocene material from cave and abri (rockshelter) sites is generally genetically well preserved and it was even possible to obtain genetic information from Neolithic waterlogged bones. The depositional environment is the most influential factor affecting DNA preservation. The age of the specimens also plays a crucial role, although it transpired that samples from very favourable conditions, like deep caves, can be better preserved than younger material. Under similar conditions, older samples are less well preserved and accumulated more post mortem damage derived lesion.

Within this project, for the first time all Palaeolithic and Neolithic sites with more than one remain of a certain species, the horse, and a preliminary selection of Iron Age and Roman time samples have been screened for DNA preservation in Switzerland. The outcome of this test is very promising and applicable to other species and further investigations of demographic developments and phenotypic characteristics. The continuous augmentation of data from different depositional contexts and periods is warranted.

Secondly, mtDNA d-loop variation of Pleistocene and Early Holocene wild horse populations was investigated and put in context with palaeoclimatological, palaeoenvironmental and archaeological data. For Switzerland, a discontinuous population history within the last 50 k years was described. The demographic development, an expansion after the LGM, was in disagreement to the development in other parts of Eurasia, particularly north eastern Asia, where abundance peaked during the LGM and decreased from then on. The yet low sample sizes from the transition time Late Pleistocene/Early Holocene allows only tentative speculating on the local dispersal/replacement/extinction pattern of wild horses. Beside the methodological challenges due to the discontinuous and unbalanced representation of equid sequences this analysis provided the first comprehensive investigation of wild horse remains from one restricted region. This approach has offered the opportunity to focus on aspects of horse population development that might be overlooked in the global picture by demonstrating sensitive reaction patterns to changing environmental conditions. To draw a picture of Eurasian horse demographic development, it is necessary to follow a comprehensive geographically and chronologically dense sampling approach, comparable to the Swiss example.

Thirdly, we examined mtDNA d-loop variation and coat colour of Iron Age domestic horses. A possible genetic differentiation of morphologically different animals was enquired via matrilinear diversity, and the investigation of coat colouration served to detect phenotypical noticeable individuals and to relate their incidence to the archaeological context. Female lineages were highly diverse, yet coat colourations were limited to the basic colours bay,

black and chestnut. The few larger horses in our dataset were not genetically distinct from the smaller majority. Breeding interests were apparently focussed on good performance and low maintenance horses, and to avoid inbreeding. This emphasises the skill and expertise Celtic stock keepers executed on this highly valuable and prestigious animal.

The lineage diversity present in Iron Age Switzerland does not differ from (roughly) contemporaneous variation in the rest of Europe except for Iberia and is particularly similar to eastern Europe. This finding supports the hypothesis, based on archaeological evidence of e.g. eastern European type bridle fragments, of an eastern origin of Swiss domestic horses. A contribution of local wild mares to the domestic gene pool can be ranked low, not least because they were most probably on the edge of extinction by the 3rd millennium BC.

The contextualisation of Swiss wild and ancient domestic horses with the Eurasian variation revealed that, at our current state of knowledge, domestics had higher nucleotide diversity than wild horses. This observation contradicts the general ideas of domestication (bottleneck) and of the velocity of mutation rates (within 5 ka since domestication started). Predictably this ratio will be adjusted as more pre-domestic specimens from Eurasia will be sequenced. Recent technical progress and leaps in the understanding of wild horse genetics (Orlando *et al.*, 2013, Schubert *et al.*, 2014, Imsland *et al.*, 2016) as well as regional approaches to chronological genetic investigations as presented here, are heralding fascinating new insights into the evolution and history of a species as much appreciated as the horse.

6. Bibliography

Achilli A, Olivieri A, Soares P, Lancioni H, Hooshiar Kashani B, Perego UA, Nergadze SG, Carossa V, Santagostino M, Capomaccio S, Felicetti M, Al-Achkar W, Penedo MC, Verini-Supplizi A, Houshmand M, Woodward SR, Semino O, Silvestrelli M, Giulotto E, Pereira L, Bandelt HJ and Torroni A, 2012. Mitochondrial genomes from modern horses reveal the major haplogroups that underwent domestication, Proc Natl Acad Sci U S A 109, 2449-2454. doi: 10.1073/pnas.1111637109.

Adler CJ, Haak W, Donlon D and Cooper A, 2011. Survival and recovery of DNA from ancient teeth and bones, J Archaeol Sci 38, 956-964. doi: 10.1016/j.jas.2010.11.010.

Affolter J, Cattin M-I, Leesch D, Morel P, Plumettaz N, Thew N and Wendling G, 1997. Monruz - Une nouvelle station magdalénienne au bord du lac de Neuchâtel, Archäologie Schweiz 17, 94-104.

Albrecht G, 1982. Rezension von Bandi HG et al. (1977): Die Kultur der Eiszeitjäger aus dem Kesslerloch, Jahrbuch der Schweizer Gesellschaft für Ur- und Frühgeschichte 65, 239-240.

Albrecht MC and Schoenen D, 2003. Die Verwesung, Verein WaBoLu, Berlin.

Allentoft ME, Collins M, Harker D, Haile J, Oskam CL, Hale ML, Campos PF, Samaniego JA, Gilbert MT, Willerslev E, Zhang G, Scofield RP, Holdaway RN and Bunce M, 2012. The half-life of DNA in bone: measuring decay kinetics in 158 dated fossils, Proc Biol Sci 279, 4724-4733. doi: 10.1098/rspb.2012.1745.

Ammann B, van Leeuwen JFN, van der Knaap WO, Lischke H, Heiri O and Tinner W, 2013. Vegetation responses to rapid warming and to minor climatic fluctuations during the Late-Glacial Interstadial (GI-1) at Gerzensee (Switzerland), Palaeogeogr, Palaeoclimatol, Palaeoecol 391, 40-59. doi: 10.1016/j.palaeo.2012.07.010.

Anderson CN, Ramakrishnan U, Chan YL and Hadly EA, 2005. Serial SimCoal: a population genetics model for data from multiple populations and points in time, Bioinformatics 21, 1733-1734. doi: 10.1093/bioinformatics/bti154.

Anthony DW, 2007. The horse, the wheel, and language. How Bronze Age riders from the Eurasian steppes shaped the modern world, Princeton University Press, Princeton.

Arbogast RM, Benoit C, Lepetz S, Méniel P and Yvinec J-H, 2002. Archéologie du cheval. Des origines à la période moderne en France, Hespérides, Paris.

Bailey E, 2015. Genetics After Twilight, J Equine Vet Sci 35, 361-366. doi: 10.1016/j.jevs.2015.03.198.

Bally E, 1908. Höhlenfunde im sog. Käsloch (Kt. Solothurn), Anzeiger für schweizerische Altertumskunde 10, 1-12.

Bandi HG, 1967/68. Untersuchung eines Felsschutzdaches bei Neumühle, Gemeinde Pleigne, Kt. Bern, Jahrbuch des Bernschen historischen Museums 47/48, 95-113.

Bandi HG, Lüdin C, Mamber W, Schaub S, Schmid E and Welten M, 1952/53. Die Brügglihöhle an der Kohlholzhalde bei Nenzlingen (Kt. Bern), eine neue Fundstelle des Spätmagdalénien im untern Birstal, Jahrbuch des Bernschen historischen Museums 32/33, 45-76.

Barnett R, Shapiro B, Barnes I, Ho SY, Burger J, Yamaguchi N, Higham TF, Wheeler HT, Rosendahl W, Sher AV, Sotnikova M, Kuznetsova T, Baryshnikov GF, Martin LD, Harington CR, Burns JA and Cooper A, 2009. Phylogeography of lions (*Panthera leo* ssp.) reveals three distinct taxa and a late Pleistocene reduction in genetic diversity, Mol Ecol 18, 1668-1677. doi: 10.1111/j.1365-294X.2009.04134.x.

Barr JH, 1977. Die Rislisberghöhle, ein neuer Magdalénien-Fundplatz im Schweizer Jura, Archäologisches Korrespodenzblatt 7, 85-87.

Bellone RR, 2010. Pleiotropic effects of pigmentation genes in horses, Anim Genet 41 Suppl 2, 100-110. doi: 10.1111/j.1365-2052.2010.02116.x.

Bernicchia A, Fugazzola MA, Gemelli V, Mantovani B, Lucchetti A, Cesari M and Speroni E, 2006. DNA recovered and sequenced from an almost 7000 y-old Neolithic polypore, *Daedaleopsis tricolor*, Mycol Res 110, 14-17. doi: 10.1016/j.mycres.2005.09.012.

Bigi D, Perrotta G and Zambonelli P, 2014. Genetic analysis of seven Italian horse breeds based on mitochondrial DNA D-loop variation, Anim Genet 45, 593-595. doi: 10.1111/age.12156.

Bollongino R, Burger J, Powell A, Mashkour M, Vigne JD and Thomas MG, 2012. Modern taurine cattle descended from small number of near-eastern founders, Mol Biol Evol 29, 2101-2104. doi: 10.1093/molbev/mss092.

Bollongino R, Tresset A and Vigne J-D, 2008. Environment and excavation: Pre-lab impacts on ancient DNA analyses, C R Palevol 7, 91-98. doi: 10.1016/j.crpv.2008.02.002.

Bosinski G, 1967. Die mittelpaläolithischen Funde im Westlichen Mitteleuropa, Köln, Graz, Böhlau.

Bower MA, Campana MG, Whitten M, Edwards CJ, Jones H, Barrett E, Cassidy R, Nisbet RE, Hill EW, Howe CJ and Binns M, 2011. The cosmopolitan maternal heritage of the Thoroughbred racehorse breed shows a significant contribution from British and Irish native mares, Biol Lett 7, 316-320. doi: 10.1098/rsbl.2010.0800.

Bower MA, Edwards CJ, Evans C and Downes CS, 2013. Horse husbandry in Iron Age and Romano-British East Anglia: ancient DNA analysis of horses at Langdale Hale, Cambridgeshire, in: Evans C, Appleby G, Lucy S and Regan R (Eds.), Process and history : Romano-British communities at Colne Fen, Earith: an inland port and supply farm, University Press, Cambridge, pp. 140-143.

Brace S, Palkopoulou E, Dalen L, Lister AM, Miller R, Otte M, Germonpre M, Blockley SP, Stewart JR and Barnes I, 2012. Serial population extinctions in a small mammal indicate Late Pleistocene ecosystem instability, Proc Natl Acad Sci U S A 109, 20532-20536. doi: 10.1073/pnas.1213322109.

Brandt G, Haak W, Adler CJ, Roth C, Szecsenyi-Nagy A, Karimnia S, Möller-Rieker S, Meller H, Ganslmeier R, Friederich S, Dresely V, Nicklisch N, Pickrell JK, Sirocko F, Reich D, Cooper A, Alt KW and Genographic C, 2013. Ancient DNA reveals key stages in the formation of central European mitochondrial genetic diversity, Science 342, 257-261. doi: 10.1126/science.1241844.

Briggs AW, Stenzel U, Johnson PL, Green RE, Kelso J, Prüfer K, Meyer M, Krause J, Ronan MT, Lachmann M and Paabo S, 2007. Patterns of damage in genomic DNA sequences from a Neandertal, Proc Natl Acad Sci U S A 104, 14616-14621. doi: 10.1073/pnas.0704665104.

Brombacher C and Kühn M, 2005. Vegetationsentwicklung und Umland der Siedlungen, in: Windler R, Marti R, Niffeler U and Steiner L (Eds.), SPM VI: Frühmittelalter, Verlag Schweizerische Gesellschaft für Ur- und Frühgeschichte, Basel, pp. 88-90.

Brotherton P, Endicott P, Sanchez JJ, Beaumont M, Barnett R, Austin J and Cooper A, 2007. Novel high-resolution characterization of ancient DNA reveals C > U-type base modification events as the sole cause of post mortem miscoding lesions, Nucleic Acids Res 35, 5717-5728. doi: 10.1093/nar/gkm588.

Brunaux J-L, 1997. Les sanctuaires celtiques de Gournay-sur-Aronde et de Ribemont-sur-Ancre, une nouvelle approche de la religion gauloise, Comptes-rendus des séances de l'année... - Académie des inscriptions et belles-lettres 141, 567-600. doi: 10.3406/crai.1997.15761.

Budiansky S, 1997. The nature of horses, their evolution, intelligence and behavior, Weidenfeld & Nicolson, London.

Bullinger J, Leesch D and Plumettaz N, 2006. Le site magdalénien de Monruz, 1: Premiers éléments pour l'analyse d'un habitat de plein air, Archéologie neuchâteloise, Service et musée cantonal d'Archéologie, Neuchâtel.

Bundesamt für Meteorologie und Klimatologie MeteoSchweiz BfMuKM.

Bundesamt für Raumentwicklung ARE BfRA.

Buoncristiani JF and Campy M, 2004. Expansion and retreat of the Jura ice sheet (France) during the last glacial maximum, Sediment Geol 165, 253-264. doi: 10.1016/j.sedgeo.2003.11.007.

Cai D, Tang Z, Han L, Speller CF, Yang DY, Ma X, Cao Je, Zhu H and Zhou H, 2009. Ancient DNA provides new insights into the origin of the Chinese domestic horse, J Archaeol Sci 36, 835-842. doi: 10.1016/j.jas.2008.11.006.

Cai DW, Han L, Xie CZ, Li SN, Zhou H and Zhu H, 2007. Mitochondrial DNA analysis of Bronze Age horses recovered from Chifeng region, Inner Mongolia, China, Prog Nat Sci 17, 544-550.

Campana MG, Stock F, Barrett E, Benecke N, Barker GW, Seetah K and Bower MA, 2012. Genetic stability in the Icelandic horse breed, Anim Genet 43, 447-449. doi: 10.1111/j.1365-2052.2011.02266.x.

Campana MG, Whitten CM, Edwards CJ, Stock F, Murphy AM, Binns MM, Barker GW and Bower MA, 2010. Accurate determination of phenotypic information from historic thoroughbred horses by single base extension, PLoS ONE 5, e15172. doi: 10.1371/journal.pone.0015172.

Campos PF, Craig OE, Turner-Walker G, Peacock E, Willerslev E and Gilbert MT, 2012. DNA in ancient bone - where is it located and how should we extract it?, Ann Anat 194, 7-16. doi: 10.1016/j.aanat.2011.07.003.

Campos PF, Kristensen T, Orlando L, Sher A, Kholodova MV, Gotherstrom A, Hofreiter M, Drucker DG, Kosintsev P, Tikhonov A, Baryshnikov GF, Willerslev E and Gilbert MT, 2010a. Ancient DNA sequences point to a large loss of mitochondrial genetic diversity in the saiga antelope (*Saiga tatarica*) since the Pleistocene, Mol Ecol 19, 4863-4875. doi: 10.1111/j.1365-294X.2010.04826.x.

Campos PF, Willerslev E, Sher A, Orlando L, Axelsson E, Tikhonov A, Aaris-Sorensen K, Greenwood AD, Kahlke RD, Kosintsev P, Krakhmalnaya T, Kuznetsova T, Lemey P, MacPhee R, Norris CA, Shepherd K, Suchard MA, Zazula GD, Shapiro B and Gilbert MT, 2010b. Ancient DNA analyses exclude humans as the driving force behind late Pleistocene musk ox (*Ovibos moschatus*) population dynamics, Proc Natl Acad Sci U S A 107, 5675-5680. doi: 10.1073/pnas.0907189107.

Charif D and Lobry JR, 2007. SeqinR 1.0-2: a contributed package to the R project for statistical computing devoted to biological sequences retrieval and analysis, in: Bastolla U, Porto MJ, Roman HE and Vendruscolo M (Eds.), Structural approaches to sequence evolution: molecules, networks, populations, Springer, New York, pp. 207-232.

Cieslak M, Pruvost M, Benecke N, Hofreiter M, Morales A, Reissmann M and Ludwig A, 2010. Origin and history of mitochondrial DNA lineages in domestic horses, PLoS ONE 5, e15311. doi: 10.1371/journal.pone.0015311.

Collins MJ, Penkman KE, Rohland N, Shapiro B, Dobberstein RC, Ritz-Timme S and Hofreiter M, 2009. Is amino acid racemization a useful tool for screening for ancient DNA in bone?, Proc Biol Sci 276, 2971-2977. doi: 10.1098/rspb.2009.0563.

Coope GR, 2007. Coleoptera from the 2003 excavations of the mammoth skeleton at Niederweningen, Switzerland, Quatern Int 164-165, 130-138. doi: 10.1016/j.quaint.2006.10.004.

Coope GR and Elias SA, 2000. The environment of Upper Palaeolithic (Magdalenian and Azilian) hunters at Hauterive-Champrèveyres, Neuchâtel, Switzerland, interpreted from coleopteran remains, J Quatern Sci 15, 157-175. doi: 10.1002/(sici)1099-1417(200002)15:2<157::aid-jqs478>3.0.co;2-k.

Cupillard C, Magny M, Bocherens H, Bridault A, Bégeot C, Bichet V, Bossuet G, Drucker DG, Gauthier E, Jouannic G, Millet L, Richard H, Rius D, Ruffaldi P and Walter-Simonnet A-V, 2015. Changes in ecosystems, climate and societies in the Jura Mountains between 40 and 8 ka cal BP, Quatern Int 378, 40-72. doi: 10.1016/j.quaint.2014.05.032.

Dabney J, Meyer M and Pääbo S, 2013. Ancient DNA damage, Cold Spring Harb Perspect Biol 5. doi: 10.1101/cshperspect.a012567.

Danzeglocke U, Jöris O and Weniger B, 2012. CalPal-2007 online.

Deagle BE, Eveson JP and Jarman SN, 2006. Quantification of damage in DNA recovered from highly degraded samples-a case study on DNA in faeces, Front Zool 3, 11. doi: 10.1186/1742-9994-3-11.

Der Sarkissian C, Ermini L, Schubert M, Yang MA, Librado P, Fumagalli M, Jonsson H, Bar-Gal GK, Albrechtsen A, Vieira FG, Petersen B, Ginolhac A, Seguin-Orlando A, Magnussen K, Fages A, Gamba C, Lorente-Galdos B, Polani S, Steiner C, Neuditschko M, Jagannathan V, Feh C, Greenblatt CL, Ludwig A, Abramson NI, Zimmermann W, Schafberg R, Tikhonov A, Sicheritz-Ponten T, Willerslev E, Marques-Bonet T, Ryder OA, McCue M, Rieder S, Leeb T, Slatkin M and Orlando L, 2015. Evolutionary genomics and conservation of the endangered Przewalski's horse, Curr Biol 25, 2577-2583. doi: 10.1016/j.cub.2015.08.032.

Drescher-Schneider R, Jacquat C and Schoch W, 2007. Palaeobotanical investigations at the mammoth site of Niederweningen (Kanton Zürich), Switzerland, Quatern Int 164-165, 113-129. doi: 10.1016/j.quaint.2006.11.016.

Drucker DG, Bridault A, Cupillard C, Hujic A and Bocherens H, 2011. Evolution of habitat and environment of red deer (*Cervus elaphus*) during the Late-glacial and early Holocene in eastern

France (French Jura and the western Alps) using multi-isotope analysis ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{34}S$) of archaeological remains, Quatern Int 245, 268-278. doi: 10.1016/j.quaint.2011.07.019.
Drummond AJ, Ho SY, Phillips MJ and Rambaut A, 2006. Relaxed phylogenetics and dating with confidence, PLoS Biol 4, e88. doi: 10.1371/journal.pbio.0040088.
Drummond AJ, Rambaut A, Shapiro B and Pybus OG, 2005. Bayesian coalescent inference of past population dynamics from molecular sequences, Mol Biol Evol 22, 1185-1192. doi: 10.1093/molbev/msi103.
Drummond AJ, Suchard MA, Xie D and Rambaut A, 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7, Mol Biol Evol 29, 1969-1973. doi: 10.1093/molbev/mss075.
Edwards CJ, Bollongino R, Scheu A, Chamberlain A, Tresset A, Vigne JD, Baird JF, Larson G, Ho SY, Heupink TH, Shapiro B, Freeman AR, Thomas MG, Arbogast RM, Arndt B, Bartosiewicz L, Benecke N, Budja M, Chaix L, Choyke AM, Coqueugniot E, Dohle HJ, Goldner H, Hartz S, Helmer D, Herzig B, Hongo H, Mashkour M, Özdogan M, Pucher E, Roth G, Schade-Lindig S, Schmölcke U, Schulting RJ, Stephan E, Uerpmann HP, Voros I, Voytek B, Bradley DG and Burger J, 2007. Mitochondrial DNA analysis shows a Near Eastern Neolithic origin for domestic cattle and no indication of domestication of European aurochs, Proc Biol Sci 274, 1377-1385. doi: 10.1098/rspb.2007.0020.
Edwards CJ, Magee DA, Park SD, McGettigan PA, Lohan AJ, Murphy A, Finlay EK, Shapiro B, Chamberlain AT, Richards MB, Bradley DG, Loftus BJ and MacHugh DE, 2010. A complete mitochondrial genome sequence from a mesolithic wild aurochs (*Bos primigenius*), PLoS ONE 5, e9255. doi: 10.1371/journal.pone.0009255.
Egloff M, 1985. Fouilles de Champréveyres, Musée Cantonal d'Archéologie, 109-119.
Egloff M, 1991. Rapport Annuel, Musée cantonal d'Archéologie
Elsner J, Hofreiter M, Schibler J and Schlumbaum A, 2017. Ancient mtDNA diversity reveals specific population development of wild horses in Switzerland after the Last Glacial Maximum, PLoS ONE 12, e0177458. doi: 10.1371/journal.pone.0177458.
Erren M, 1985-2003. P. Vergilius Maro: Georgica, Winter, Heidelberg.
Excoffier L and Lischer HE, 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows, Mol Ecol Resour 10, 564-567. doi: 10.1111/j.1755-0998.2010.02847.x.
Fernandez H, Taberlet P, Mashkour M, Vigne J-D and Luikart G, 2005. Assessing the origin and diffusion of domestic goats using ancient DNA, in: Vigne J-D, Peters J and Helmer D (Eds.), First steps of animal domestication - New archaeozoological approaches, Oxbow Books, Oxford, pp. 50-54.
Fortes GG, Speller CF, Hofreiter M and King TE, 2013. Phenotypes from ancient DNA: approaches, insights and prospects, Bioessays 35, 690-695. doi: 10.1002/bies.201300036.
Fu Q, Mittnik A, Johnson PLF, Bos K, Lari M, Bollongino R, Sun C, Giemsch L, Schmitz R, Burger J, Ronchitelli AM, Martini F, Cremonesi RG, Svoboda J, Bauer P, Caramelli D, Castellano S, Reich D, Pääbo S and Krause J, 2013. A revised timescale for human evolution based on ancient mitochondrial genomes, Curr Biol 23, 553-559. doi: 10.1016/j.cub.2013.02.044.
Furger R, 1977. Die mittelpaläolithische Station beim untern Steinbruch von Münchenstein BL, Regio Basilensis, 58-72.
Geigl EM, 2002. On the circumstances surrounding the preservation and analysis of very old DNA, Archaeometry 44, 337-342.
Gerbault P, Leonardi M, Powell A, Weber C, Benecke N, Burger J and Thomas MG, 2012. Domestication and migrations: Using mitochondrial DNA to infer domestication processes of goats and horses, in: Kaiser E, Burger J and Schier WO (Eds.), Population dynamics in Prehistory and Early History. New approaches using stable isotopes and genetics, de Gruyter, Berlin, pp. 17-30.
Gilbert MT, Bandelt HJ, Hofreiter M and Barnes I, 2005. Assessing ancient DNA studies, Trends Ecol Evol 20, 541-544. doi: 10.1016/j.tree.2005.07.005.
Gilbert MT, Binladen J, Miller W, Wiuf C, Willerslev E, Poinar H, Carlson JE, Leebens-Mack JH and Schuster SC, 2007. Recharacterization of ancient DNA miscoding lesions: insights in the era of sequencing-by-synthesis, Nucleic Acids Res 35, 1-10. doi: 10.1093/nar/gkl483.

Gilbert MT, Janaway RC, Tobin DJ, Cooper A and Wilson AS, 2006. Histological correlates of post mortem mitochondrial DNA damage in degraded hair, Forensic Sci Int 156, 201-207. doi: 10.1016/j.forsciint.2005.02.019.

Girdland Flink L, Allen R, Barnett R, Malmström H, Peters J, Eriksson J, Andersson L, Dobney K and Larson G, 2014. Establishing the validity of domestication genes using DNA from ancient chickens, Proc Natl Acad Sci U S A 111, 6184-6189. doi: 10.1073/pnas.1308939110.

Götherström A, Collins MJ, Angerbjörn A and Liden K, 2002. Bone preservation and DNA amplification, Archaeometry 44, 395-404. doi: 10.1111/1475-4754.00072.

Gravlund P, Aaris-Sorensen K, Hofreiter M, Meyer M, Bollback JP and Noe-Nygaard N, 2012. Ancient DNA extracted from Danish aurochs (*Bos primigenius*): genetic diversity and preservation, Ann Anat 194, 103-111. doi: 10.1016/j.aanat.2011.10.011.

Guarino FM, Angelini F, Odierna G, Bianco MR, Bernardo GD, Forte A, Cascino A and Cipollaro M, 2009. Detection of DNA in ancient bones using histochemical methods, Biotechnic & Histochemistry 75, 110-117. doi: 10.3109/10520290009066488.

Guthrie RD, 2001. Origin and causes of the mammoth steppe: a story of cloud cover, woolly mammal tooth pits, buckles, and inside-out Beringia, Quatern Sci Rev 20, 549-574.

Haak W, Lazaridis I, Patterson N, Rohland N, Mallick S, Llamas B, Brandt G, Nordenfelt S, Harney E, Stewardson K, Fu Q, Mittnik A, Banffy E, Economou C, Francken M, Friederich S, Pena RG, Hallgren F, Khartanovich V, Khokhlov A, Kunst M, Kuznetsov P, Meller H, Mochalov O, Moiseyev V, Nicklisch N, Pichler SL, Risch R, Rojo Guerra MA, Roth C, Szecsenyi-Nagy A, Wahl J, Meyer M, Krause J, Brown D, Anthony D, Cooper A, Alt KW and Reich D, 2015. Massive migration from the steppe was a source for Indo-European languages in Europe, Nature 522, 207-211. doi: 10.1038/nature14317.

Haas C, Shved N, Ruhli FJ, Papageorgopoulou C, Purps J, Geppert M, Willuweit S, Roewer L and Krawczak M, 2013. Y-chromosomal analysis identifies the skeletal remains of Swiss national hero Jörg Jenatsch (1596-1639), Forensic Sci Int Genet 7, 610-617. doi: 10.1016/j.fsigen.2013.08.006.

Hahn J, 1983. Eiszeitliche Jäger zwischen 35000 und 15000 vor heute, in: Müller-Beck H (Ed.), Urgeschichte in Baden-Württemberg, Konrad Theiss, Stuttgart, pp. 273-330.

Haile J, Froese DG, Macphee RD, Roberts RG, Arnold LJ, Reyes AV, Rasmussen M, Nielsen R, Brook BW, Robinson S, Demuro M, Gilbert MT, Munch K, Austin JJ, Cooper A, Barnes I, Moller P and Willerslev E, 2009. Ancient DNA reveals late survival of mammoth and horse in interior Alaska, Proc Natl Acad Sci U S A 106, 22352-22357. doi: 10.1073/pnas.0912510106.

Hajdas I, Bonani G, Furrer H, Mäder A and Schoch W, 2007. Radiocarbon chronology of the mammoth site at Niederweningen, Switzerland: Results from dating bones, teeth, wood, and peat, Quatern Int 164-165, 98-105. doi: 10.1016/j.quaint.2006.10.007.

Hall T, 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, Nucleic Acids Symposium Series 41, 95-98.

Hansen AJ, Mitchell DL, Wiuf C, Paniker L, Brand TB, Binladen J, Gilichinsky DA, Ronn R and Willerslev E, 2006. Crosslinks rather than strand breaks determine access to ancient DNA sequences from frozen sediments, Genetics 173, 1175-1179. doi: 10.1534/genetics.106.057349.

Haynes S, Searle JB, Bretman A and Dobney KM, 2002. Bone preservation and ancient DNA: The application of screening methods for predicting DNA survival, J Archaeol Sci 29, 585-592. doi: 10.1006/jasc.2001.0731.

Heierli J, 1907. Das Kesslerloch bei Thaingen, Kommissions-Verlag von Georg & Co, Basel.

Heiri O, Koinig KA, Spötl C, Barrett S, Brauer A, Drescher-Schneider R, Gaar D, Ivy-Ochs S, Kerschner H, Luetscher M, Moran A, Nicolussi K, Preusser F, Schmidt R, Schoeneich P, Schwörer C, Sprafke T, Terhorst B and Tinner W, 2014. Palaeoclimate records 60–8 ka in the Austrian and Swiss Alps and their forelands, Quatern Sci Rev 106, 186-205. doi: 10.1016/j.quascirev.2014.05.021.

Heyn P, 2010. Road blocks on paleogenomes - Polymerase extension profiling reveals the frequency of blocking lesions in ancient DNA, Nucleic Acids Res 38, e161.

Higgins D and Austin JJ, 2013. Teeth as a source of DNA for forensic identification of human remains: a review, Sci Justice 53, 433-441. doi: 10.1016/j.scijus.2013.06.001.

Hill EW, Bradley DG, Al-Barody M, Ertugrul O, Splan RK, Zakharov I and Cunningham EP, 2002. History and integrity of thoroughbred dam lines revealed in equine mtDNA variation, Anim Genet 33, 287-294.
Hofreiter M, Serre D, Poinar HN, Kuch M and Pääbo S, 2001. Ancient DNA, Nat Rev Genet 2, 353-359. doi: 10.1038/35072071.
Hoke N, Burger J, Weber C, Benecke N, Grupe G and Harbeck M, 2011. Estimating the chance of success of archaeometric analyses of bone: UV-induced bone fluorescence compared to histological screening, Palaeogeogr, Palaeoclimatol, Palaeoecol 310, 23-31. doi: 10.1016/j.palaeo.2011.03.021.
Höneisen M and Peyer S, 1994. Schweizersbild – ein Jägerlager der Späteiszeit: Beiträge und Dokumente zur Ausgrabung vor 100 Jahren, Kantonsarchäologie, Schaffhausen.
Hoss M, 1995. Ancient DNA, Horm Res 43, 118-120. doi: 10.1159/000184253.
Housley R, Gamble C, Street M and Pettitt P, 1997. Radiocarbon evidence for the Lateglacial human recolonisation of Northern Europe, Proc Prehist Soc 63, 25-54.
Hu Q, Liu Y, Yi S and Huang D, 2015. A comparison of four methods for PCR inhibitor removal, Forensic Sci Int Genet 16, 94-97. doi: 10.1016/j.fsigen.2014.12.001.
Huber A, 1963. 1000 Jahre Pferdezucht Kloster Einsiedeln, EDE-Verlag, Einsiedeln.
Hughes PD, Gibbard PL and Ehlers J, 2013. Timing of glaciation during the last glacial cycle: evaluating the concept of a global 'Last Glacial Maximum' (LGM), Earth-Sci Rev 125, 171-198. doi: 10.1016/j.earscirev.2013.07.003.
Hüster-Plogmann H and Rehazek A, 2005. Landschaftsentwicklung aus archäozoologischer Sicht, in: Windler R, Marti R, Niffeler U and Steiner L (Eds.), SPM VI: Frühmittelalter, Verlag Schweizerische Gesellschaft für Ur- und Frühgeschichte, Basel, pp. 91-92.
Immel A, Drucker DG, Bonazzi M, Jahnke TK, Münzel SC, Schuenemann VJ, Herbig A, Kind CJ and Krause J, 2015. Mitochondrial genomes of giant deers suggest their late survival in Central Europe, Sci Rep 5, 10853. doi: 10.1038/srep10853.
Imsland F, McGowan K, Rubin CJ, Henegar C, Sundstrom E, Berglund J, Schwochow D, Gustafson U, Imsland P, Lindblad-Toh K, Lindgren G, Mikko S, Millon L, Wade C, Schubert M, Orlando L, Penedo MC, Barsh GS and Andersson L, 2016. Regulatory mutations in TBX3 disrupt asymmetric hair pigmentation that underlies Dun camouflage color in horses, Nat Genet 48, 152-158. doi: 10.1038/ng.3475.
Ivy-Ochs S, Kerschner H, Reuther A, Preusser F, Heine K, Maisch M, Kubik PW and Schlüchter C, 2008. Chronology of the last glacial cycle in the European Alps, J Quatern Sci 23, 559-573. doi: 10.1002/jqs.1202.
Ivy-Ochs S, Schäfer J, Kubik PW, Synal H-A and Schlüchter C, 2004. Timing of deglaciation on the northern Alpine foreland (Switzerland), Eclogae Geol Helv 97, 47-55. doi: 10.1007/s00015-004-1110-0.
Jacomet S, Jacquat C, Maise C, Schibler J, Stopp B, Studer J, Wick L and Winter M, 1999. Klima, Umwelt, Landwirtschaft und Ernährung, in: Müller F, Kaenel G and Lüscher G (Eds.), SPM IV: Eisenzeit, Verlag Schweizerische Gesellschaft für Ur- und Frühgeschichte, Basel, pp. 93-136.
Jacomet S, Magny M and Burga CA, 1995. Klima- und Seespiegelschwankungen im Verlauf des Neolithikums und ihre Auswirkungen auf die Besiedlung der Seeufer, in: Stöckli WE, Niffeler U and Gross-Klee E (Eds.), SPM II: Neolithikum, Verlag Schweizerische Gesellschaft für Ur- und Frühgeschichte, Basel, pp. 53-58.
Jansen T, Forster P, Levine MA, Oelke H, Hurles M, Renfrew C, Weber J and Olek K, 2002. Mitochondrial DNA and the origins of the domestic horse, Proc Natl Acad Sci U S A 99, 10905-10910. doi: 10.1073/pnas.152330099.
Jobling MA, Hurles ME and Tyler-Smith C, 2004. Human evolutionary genetics, Garland Publishing.
Johnstone CJ, 2004. A biometric study of equids in the Roman world, PhD thesis Archaeology department, University of York, York.
Jost-Stauffer M, Coope GR and Schlüchter C, 2001. A coleopteran fauna from the middle Würm (Weichselian) of Switzerland and its bearing on palaeobiogeography, palaeoeclimate and palaeoecology, J Quatern Sci 16, 257-268. doi: 10.1002/jqs.616.

Jost-Stauffer M, Coope GR and Schlüchter C, 2005. Environmental and climatic reconstructions during Marine Oxygen Isotope Stage 3 from Gossau, Swiss Midlands, based on coleopteran assemblages, Boreas 34, 53-60. doi: 10.1080/03009480510012836.
Jung Y-H, Han S-H, Shin T and Oh M-Y, 2002. Genetic characterization of horse bone excavated from the Kwakiji archaeological site, Jeju, Korea, Mol Cells 14, 224-230.
Junkelmann M, 1991. Die Reiter Roms. Teil II: Der militärische Einsatz, Philipp von Zabern, Mainz.
Kass RE and Raftery AE, 1995. Bayes Factors, J Am Stat Assoc 90, 773-795. doi: 10.1080/01621459.1995.10476572.
Keane T, Creevey C, Pentony M, Naughton T and Mclnerney J, 2006. Assessment of methods for amino acid matrix selection and their use on empirical data shows that ad hoc assumptions for choice of matrix are not justified, BMC Evol Biol 6, 29.
Kelly C, 2008. Attila the Hun, Barbarian terror and the fall of the Roman Empire, Bodley Head, London.
Kemp BM, Monroe C, Judd KG, Reams E and Grier C, 2014. Evaluation of methods that subdue the effects of polymerase chain reaction inhibitors in the study of ancient and degraded DNA, J Archaeol Sci 42, 373-380. doi: 10.1016/j.jas.2013.11.023.
Keyser-Tracqui C, Blandin-Frappin P, Francfort HP, Ricaut FX, Lepetz S, Crubezy E, Samashev Z and Ludes B, 2005. Mitochondrial DNA analysis of horses recovered from a frozen tomb (Berel site, Kazakhstan, 3rd Century BC), Anim Genet 36, 203-209. doi: 10.1111/j.1365-2052.2005.01316.x.
Krause-Kyora B, Makarewicz C, Evin A, Flink LG, Dobney K, Larson G, Hartz S, Schreiber S, von Carnap-Bornheim C, von Wurmb-Schwark N and Nebel A, 2013. Use of domesticated pigs by Mesolithic hunter-gatherers in northwestern Europe, Nat Commun 4, 2348. doi: 10.1038/ncomms3348.
Kunst GK, 2000. Archaeozoological evidence for equid use, sex structure and mortality in a Roman auxiliary fort (Carnuntum-Petronell, Lower Austria), Anthropozoologica 31, 109-118.
Lambert J, Winter T, Dewez T and Sabourault P, 2005. New hypotheses on the maximum damage area of the 1356 Basel earthquake (Switzerland), Quatern Sci Rev 24, 381-399. doi: 10.1016/j.quascirev.2004.02.019.
Lamers R, Hayter S and Matheson CD, 2009. Postmortem miscoding lesions in sequence analysis of human ancient mitochondrial DNA, J Mol Evol 68, 40-55. doi: 10.1007/s00239-008-9184-3.
Lari M, Di Vincenzo F, Borsato A, Ghirotto S, Micheli M, Balsamo C, Collina C, De Bellis G, Frisia S, Giacobini G, Gigli E, Hellstrom JC, Lannino A, Modi A, Pietrelli A, Pilli E, Profico A, Ramirez O, Rizzi E, Vai S, Venturo D, Piperno M, Lalueza-Fox C, Barbujani G, Caramelli D and Manzi G, 2015. The Neanderthal in the karst: First dating, morphometric, and paleogenetic data on the fossil skeleton from Altamura (Italy), J Hum Evol 82, 88-94. doi: 10.1016/j.jhevol.2015.02.007.
Larson G, Albarella U, Dobney K, Rowley-Conwy P, Schibler J, Tresset A, Vigne JD, Edwards CJ, Schlumbaum A, Dinu A, Balacsescu A, Dolman G, Tagliacozzo A, Manaseryan N, Miracle P, Van Wijngaarden-Bakker L, Masseti M, Bradley DG and Cooper A, 2007. Ancient DNA, pig domestication, and the spread of the Neolithic into Europe, Proc Natl Acad Sci U S A 104, 15276-15281. doi: 10.1073/pnas.0703411104.
Larson G, Liu R, Zhao X, Yuan J, Fuller D, Barton L, Dobney K, Fan Q, Gu Z, Liu XH, Luo Y, Lv P, Andersson L and Li N, 2010. Patterns of East Asian pig domestication, migration, and turnover revealed by modern and ancient DNA, Proc Natl Acad Sci U S A 107, 7686-7691. doi: 10.1073/pnas.0912264107.
Lassen C, Hummel S and Herrmann B, 2000. Molecular sex identification of stillborn and neonate individuals ("Traufkinder") from the burial site Aegerten, Anthropol Anz 58, 1-8. doi: 10.2307/29540906.
Lauwerier RCGM and Hessing WAM, 1992. Men, horses and the Miss Blanche effect: Roman horse burials in a cemetery at Kesteren, The Netherlands, Helinium 32, 78-109.
Le Douarin NM and Kalcheim C, 1999. The neural crest, Cambridge University Press, Cambridge.
Leesch D, 1993. Das späte Jungpaläolithikum. Zeitlicher Rahmen und Fundinventare, in: LeTensorer J-M and Niffeler U (Eds.), SPM I: Paläolithikum und Mesolithikum, Gesellschaft für Ur- und Frühgeschichte der Schweiz, Basel, pp. 153-164.

Leesch D, Affolter J, Beck CW and Schoch WH, 1997. Hauterive-Champréveyres, 10. Un campement magdalénien au bord du lac neuchâtel: cadre chronologique et culturel, mobilier et structures, analyse spatiale (secteur 1), Archéologie neuchâteloise, Musée cantonal d'archéologie, Neuchâtel.
Leesch D, Bullinger J, Cattin M, Müller W and Plumettaz N, 2010. Hearth and hearth-related activities in Magdalenian open-air sites: the case studies of Champréveyres and Monruz (Switzerland) and their relevance to an understanding of upper Palaeolithic site structure, The Magdalenian in central Europe. New finds and concepts, Instytut Archeologii Uniwersytetu Rzeszowskiego, Rzeszów pp. 53-69.
Leesch D and Müller W, 2012a. Neue Radiokarbondaten an Knochen, Zähnen und Geweih aus Magdalénien-Fundstellen der Schweiz und ihre Bedeutung für die Stellung des Magdalénien innerhalb des Spätglazials, Jahrbuch der Archäologie Schweiz 95, 1-12.
Leesch D, Müller W, Nielsen E and Bullinger J, 2012b. The Magdalenian in Switzerland: Re-colonization of a newly accessible landscape, Quatern Int 272-273, 191-208. doi: 10.1016/j.quaint.2012.04.010.
Lemey P, Rambaut A, Welch JJ and Suchard MA, 2010. Phylogeography takes a relaxed random walk in continuous space and time, Mol Biol Evol 27, 1877-1885. doi: 10.1093/molbev/msq067.
LeTensorer J-M and Niffeler U, 1993. Paläolithikum und Mesolithikum, Die Schweiz vom Paläolithikum bis zum frühen Mittelalter, Schweizerische Gesellschaft für Ur- und Frühgeschichte, Basel.
Lindahl T, 1993. Instability and decay of the primary structure of DNA, Nature 362, 709-715. doi: 10.1038/362709a0.
Lippold S, Knapp M, Kuznetsova T, Leonard JA, Benecke N, Ludwig A, Rasmussen M, Cooper A, Weinstock J, Willerslev E, Shapiro B and Hofreiter M, 2011a. Discovery of lost diversity of paternal horse lineages using ancient DNA, Nat Commun 2, 450. doi: 10.1038/ncomms1447.
Lippold S, Matzke NJ, Reissmann M and Hofreiter M, 2011b. Whole mitochondrial genome sequencing of domestic horses reveals incorporation of extensive wild horse diversity during domestication, BMC Evol Biol 11, 328. doi: 10.1186/1471-2148-11-328.
Lira J, Linderholm A, Olaria C, Brandstrom Durling M, Gilbert MT, Ellegren H, Willerslev E, Liden K, Arsuaga JL and Götherström A, 2010. Ancient DNA reveals traces of Iberian Neolithic and Bronze Age lineages in modern Iberian horses, Mol Ecol 19, 64-78. doi: 10.1111/j.1365-294X.2009.04430.x.
Lopes MS, Mendonca D, Cymbron T, Valera M, da Costa-Ferreira J and Machado Ada C, 2005. The Lusitano horse maternal lineage based on mitochondrial D-loop sequence variation, Anim Genet 36, 196-202. doi: 10.1111/j.1365-2052.2005.01279.x.
Lorenzen ED, Nogues-Bravo D, Orlando L, Weinstock J, Binladen J, Marske KA, Ugan A, Borregaard MK, Gilbert MT, Nielsen R, Ho SY, Goebel T, Graf KE, Byers D, Stenderup JT, Rasmussen M, Campos PF, Leonard JA, Koepfli KP, Froese D, Zazula G, Stafford TW, Jr., Aaris-Sorensen K, Batra P, Haywood AM, Singarayer JS, Valdes PJ, Boeskorov G, Burns JA, Davydov SP, Haile J, Jenkins DL, Kosintsev P, Kuznetsova T, Lai X, Martin LD, McDonald HG, Mol D, Meldgaard M, Munch K, Stephan E, Sablin M, Sommer RS, Sipko T, Scott E, Suchard MA, Tikhonov A, Willerslev R, Wayne RK, Cooper A, Hofreiter M, Sher A, Shapiro B, Rahbek C and Willerslev E, 2011. Species-specific responses of Late Quaternary megafauna to climate and humans, Nature 479, 359-364. doi: 10.1038/nature10574.
Lüdin C, 1938. Kohlerhöhle bei Brislach (Amt Laufen, Bern), Jahrbuch der Schweizerischen Gesellschaft für Ur- und Frühgeschichte 30, 61-64.
Ludwig A, Pruvost M, Reissmann M, Benecke N, Brockmann GA, Castanos P, Cieslak M, Lippold S, Llorente L, Malaspinas AS, Slatkin M and Hofreiter M, 2009. Coat color variation at the beginning of horse domestication, Science 324, 485. doi: 10.1126/science.1172750.
Ludwig A, Reissmann M, Benecke N, Bellone R, Sandoval-Castellanos E, Cieslak M, Fortes GG, Morales-Muniz A, Hofreiter M and Pruvost M, 2015. Twenty-five thousand years of fluctuating selection on leopard complex spotting and congenital night blindness in horses, Philos Trans R Soc Lond B Biol Sci 370, 20130386. doi: 10.1098/rstb.2013.0386.

Magny M, Maise C, Jacomet S and Burga CA, 1998. Klimaschwankungen im Verlauf der Bronzezeit, in: Hochuli S, Niffeler U and Rychner V (Eds.), SPM III: Bronzezeit, Verlag Schweizerische Gesellschaft für Ur- und Frühgeschichte, Basel, pp. 135-140.
Matisoo-Smith E and Horsburgh KA, 2012. DNA for archaeologists, Left Coast Press, Walnut Creek.
McGahern A, Bower MA, Edwards CJ, Brophy PO, Sulimova G, Zakharov I, Vizuete-Forster M, Levine M, Li S, MacHugh DE and Hill EW, 2006a. Evidence for biogeographic patterning of mitochondrial DNA sequences in Eastern horse populations, Anim Genet 37, 494-497. doi: 10.1111/j.1365-2052.2006.01495.x.
McGahern AM, Edwards CJ, Bower MA, Heffernan A, Park SD, Brophy PO, Bradley DG, MacHugh DE and Hill EW, 2006b. Mitochondrial DNA sequence diversity in extant Irish horse populations and in ancient horses, Anim Genet 37, 498-502. doi: 10.1111/j.1365-2052.2006.01506.x.
Meiri M, Lister AM, Collins MJ, Tuross N, Goebel T, Blockley S, Zazula GD, van Doorn N, Guthrie DR, Boeskorov GG, Baryshnikov GF, Sher A and Barnes I, 2014. Faunal record identifies Bering isthmus conditions as constraint to end-Pleistocene migration to the New World, Proc Biol Sci 281, 20132167. doi: 10.1098/rspb.2013.2167.
Méniel P, 1997. Les restes animaux et la définition des lieux de culte en Gaule septentrionale au deuxième Âge du Fer, Cahiers du Centre Gustave Glotz 8, 171-180. doi: 10.3406/ccgg.1997.1439.
Méniel P, 2014. Les restes animaux du site du Mormont. Eclépens et La Sarraz, canton de Vaud, vers 100 avant J.-C., Cahiers d'Archéologie Romande, Lausanne.
Merk C, 1875. Der Höhlenfund im Kesslerloch bey Thayngen (Kanton Schaffhausen): Originalbericht des Entdeckers, Hans Staub, Zürich.
Merk C, 1876. Excavations at the Kesslerloch near Thayngen, Switzerland: a cave of the reindeer period, Longman, Green and Co., London.
Meyer M, Fu Q, Aximu-Petri A, Glocke I, Nickel B, Arsuaga JL, Martinez I, Gracia A, de Castro JM, Carbonell E and Pääbo S, 2014. A mitochondrial genome sequence of a hominin from Sima de los Huesos, Nature 505, 403-406. doi: 10.1038/nature12788.
Meyer M, Kircher M, Gansauge MT, Li H, Racimo F, Mallick S, Schraiber JG, Jay F, Prüfer K, de Filippo C, Sudmant PH, Alkan C, Fu Q, Do R, Rohland N, Tandon A, Siebauer M, Green RE, Bryc K, Briggs AW, Stenzel U, Dabney J, Shendure J, Kitzman J, Hammer MF, Shunkov MV, Derevianko AP, Patterson N, Andres AM, Eichler EE, Slatkin M, Reich D, Kelso J and Pääbo S, 2012. A high-coverage genome sequence from an archaic Denisovan individual, Science 338, 222-226. doi: 10.1126/science.1224344.
Miller R, 2012. Mapping the expansion of the Northwest Magdalenian, Quatern Int 272-273, 209-230. doi: 10.1016/j.quaint.2012.05.034.
Milos A, Selmanovic A, Smajlovic L, Huel RL, Katzmarzyk C, Rizvic A and Parsons TJ, 2007. Success rates of nuclear short tandem repeat typing from different skeletal elements, Croat Med J 48, 486-493.
Minin VN, Bloomquist EW and Suchard MA, 2008. Smooth skyride through a rough skyline: Bayesian coalescent-based inference of population dynamics, Mol Biol Evol 25, 1459-1471. doi: 10.1093/molbev/msn090.
Mitchell D, Willerslev E and Hansen A, 2005. Damage and repair of ancient DNA, Mutat Res 571, 265-276. doi: 10.1016/j.mrfmmm.2004.06.060.
Molak M and Ho SY, 2011. Evaluating the impact of post-mortem damage in ancient DNA: a theoretical approach, J Mol Evol 73, 244-255. doi: 10.1007/s00239-011-9474-z.
Möller L, 2013. Der gallische Krieg / Gaius Iulius Ceasar, Marixverlag, Wiesbaden.
Morelli L, Useli A, Sanna D, Barbato M, Contu D, Pala M, Cancedda M and Francalacci P, 2014. Mitochondrial DNA lineages of Italian Giara and Sarcidano horses, Genet Mol Res 13, 8241-8257. doi: 10.4238/2014.October.20.1.
Müller-Lhotska UA, 1984. Das Pferd in der Schweiz. Von der Prähistorie bis zum ausgehenden Mittelalter, PhD thesis History department, University of Zürich, Zürich.

Münzel SC, Stiller M, Hofreiter M, Mittnik A, Conard NJ and Bocherens H, 2011. Pleistocene bears in the Swabian Jura (Germany): Genetic replacement, ecological displacement, extinctions and survival, Quatern Int 245, 225-237. doi: 10.1016/j.quaint.2011.03.060.
Napierala H, 2008. Die Tierknochen aus dem Kesslerloch. Neubearbeitung der paläolithischen Fauna, Baudepartement des Kantons Schaffhausen, Kantonsarchäologie, Schaffhausen.
Nationalgestüt Avenches N and Rizzoli A, 2004. Les sources de l'élevage du cheval en Suisse. Guide de recherche, Haras national, Avenches.
Newell RR, 2009. The Swabian Alb in the Later Stone Age. Illustrating the changes in subsistence and land-use-practices, M. Leidorf, Rahden.
Nielsen EH, 2009. Paläolithikum und Mesolithikum in der Zentralschweiz. Mensch und Umwelt zwischen 17 000 und 5 500 v. Chr., Archäologische Schriften, Luzern.
Niven L, 2003. Patterns of subsistence and settlement during the Aurignacian of the Swabian Jura, Germany, in: Zilhao J and d'Errico F (Eds.), The chronology of the Aurignacian and of the transitional technocomplexes: Dating, stratigraphies, cultural implications, Lisboa, pp. 199-212.
Nobis G, 1973. Zur Frage römerzeitlicher Hauspferde in Zentraleuropa, Zeitschrift für Säugetierkunde 38, 224-252.
Nüesch J, 1892. Une station préhistorique à Schweizersbild près de Schaffhouse, Archives des sciences physiques et naturelles, 541-548.
Nüesch J, Studer T and Schötensack O, 1904. Das Kesslerloch, eine Höhle aus paläolithischer Zeit: Neue Grabungen und Funde, Neue Denkschriften der Schweizerischen Naturforschenden Gesellschaft 39.
Nuviala P, Nowell G, Ughetto J and Balasse M, 2014. L'origine des chevaux du site du Mormont (canton de Vaud, Suisse): L'apport des isotopes du carbone, de l'oxygène et du strontium, in: Barral P, Guillaumet M-J, Roulière-Lambert M-J, Saracino M and Vitali D (Eds.), Les Celtes et le nord de l'Italie. Premier et second âges du fer, Société archéologique de l'Est, Vérone, pp. 603-610.
Olalde I, Allentoft ME, Sanchez-Quinto F, Santpere G, Chiang CW, DeGiorgio M, Prado-Martinez J, Rodriguez JA, Rasmussen S, Quilez J, Ramirez O, Marigorta UM, Fernandez-Callejo M, Prada ME, Encinas JM, Nielsen R, Netea MG, Novembre J, Sturm RA, Sabeti P, Marques-Bonet T, Navarro A, Willerslev E and Lalueza-Fox C, 2014. Derived immune and ancestral pigmentation alleles in a 7,000-year-old Mesolithic European, Nature 507, 225-228. doi: 10.1038/nature12960.
Olsen SL, 2006. Introduction, in: Olsen SL, Grant S, Choyke AM and Bartosiewicz L (Eds.), Horses and humans: The evolution of human-equine relationships, Archaeopress, Oxford.
Orcel A, 1978. Analyse archéologique des sediments. Méthode appliqué à une grande surface et résultats (caissons 6 et 7: civilisation de Cortaillod), Staatlicher Lehrmittelverlag, Bern.
Orlando L, Gilbert MT and Willerslev E, 2015. Reconstructing ancient genomes and epigenomes, Nat Rev Genet 16, 395-408. doi: 10.1038/nrg3935.
Orlando L, Ginolhac A, Zhang G, Froese D, Albrechtsen A, Stiller M, Schubert M, Cappellini E, Petersen B, Moltke I, Johnson PL, Fumagalli M, Vilstrup JT, Raghavan M, Korneliussen T, Malaspinas AS, Vogt J, Szklarczyk D, Kelstrup CD, Vinther J, Dolocan A, Stenderup J, Velazquez AM, Cahill J, Rasmussen M, Wang X, Min J, Zazula GD, Seguin-Orlando A, Mortensen C, Magnussen K, Thompson JF, Weinstock J, Gregersen K, Roed KH, Eisenmann V, Rubin CJ, Miller DC, Antczak DF, Bertelsen MF, Brunak S, Al-Rasheid KA, Ryder O, Andersson L, Mundy J, Krogh A, Gilbert MT, Kjaer K, Sicheritz-Ponten T, Jensen LJ, Olsen JV, Hofreiter M, Nielsen R, Shapiro B, Wang J and Willerslev E, 2013. Recalibrating *Equus* evolution using the genome sequence of an early Middle Pleistocene horse, Nature 499, 74-78. doi: 10.1038/nature12323.
Outram AK, Stear NA, Bendrey R, Olsen S, Kasparov A, Zaibert V, Thorpe N and Evershed RP, 2009. The earliest horse harnessing and milking, Science 323, 1332-1335. doi: 10.1126/science.1168594.
Overballe-Petersen S, Orlando L and Willerslev E, 2012. Next-generation sequencing offers new insights into DNA degradation, Trends Biotechnol 30, 364-368. doi: 10.1016/j.tibtech.2012.03.007.
Pääbo S, 1989. Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification, Proc Natl Acad Sci U S A 86, 1939-1943.

Pääbo S, Poinar H, Serre D, Jaenicke-Despres V, Hebler J, Rohland N, Kuch M, Krause J, Vigilant L and Hofreiter M, 2004. Genetic analyses from ancient DNA, Annu Rev Genet 38, 645-679. doi: 10.1146/annurev.genet.37.110801.143214.

Paradis E, 2010. pegas: an R package for population genetics with an integrated-modular approach, Bioinformatics 26, 419-420. doi: 10.1093/bioinformatics/btp696.

Paulus S and Uerpmann HP, 2007. Physikalisch-chemische Untersuchungen an Equidenzähnen aus Kalkriese zur Bestimmung von Knochenerhaltung und Jahreszeit der Schlacht, in: Wilbers-Rost S, Uerpmann HP, Uerpmann M, Grosskopf B and Tolksdorf-Lienemann E (Eds.), Kalkriese 3: Interdisziplinäre Untersuchungen auf dem Oberesch in Kalkriese, Archäologische Befunde und naturwissenschaftliche Begleituntersuchungen, Verlag Philipp von Zabern, Mainz, pp. 145-156.

Perrin B, 1917. Plutarch Lives, vol. V: Agesilaus and Pompey, Pelopidas and Marcellus, Loeb Classical Library, Harvard University Press, Cambridge.

Peters J, 1998. Römische Tierhaltung und Tierzucht, Marie Leidorf GmbH, Rahden/Westf.

Poinar HN, Hoss M, Bada JL and Pääbo S, 1996. Amino acid racemization and the preservation of ancient DNA, Science 272, 864-866.

Pollmann B, Jacomet S and Schlumbaum A, 2005. Morphological and genetic studies of waterlogged Prunus species from the Roman vicus Tasgetium (Eschenz, Switzerland), J Archaeol Sci 32, 1471-1480. doi: 10.1016/j.jas.2005.04.002.

Poncet PA, Boessinger M, Guillet A, Klopfenstein S, König-Bürgi D, Lüth A, Martin R, Montavon S, Obexer-Ruff G, Rieder S, Rubli S, Rüegg P and Trolliet CF, 2009. Wirtschafts-, gesellschafts- und umweltpolitische Bedeutung des Pferdes in der Schweiz ; Rapport des Observatoriums der schweizerischen Pferdebranche; Was gibt es Neues seit 2007?, Avenches.

Poncet PA, Guillet A, Jallon L, Lüth A, Martin R, Montavon S, Saunier E, Trolliet CF and Wohlfender K, 2007. Wirtschafts-, Gesellschafts- und Umweltpolitische Bedeutung des Pferdes in der Schweiz: Bericht der Arbeitsgruppe Pferdebranche, Avenches.

Preusser F and Degering D, 2007. Luminescence dating of the Niederweningen mammoth site, Switzerland, Quatern Int 164-165, 106-112. doi: 10.1016/j.quaint.2006.12.002.

Preusser F, Geyh MA and Schlüchter C, 2003. Timing of Late Pleistocene climate change in lowland Switzerland, Quatern Sci Rev 22, 1435-1445. doi: 10.1016/s0277-3791(03)00127-6.

Preusser F, Graf HR, Keller O, Krayss E and Schlüchter C, 2011. Quaternary glaciation history of northern Switzerland, Quatern Sci J 60, 282-305. doi: 10.3285/eg.60.2-3.06.

Priskin K, Szabo K, Tomory G, Bogacsi-Szabo E, Csanyi B, Eordogh R, Downes CS and Rasko I, 2010. Mitochondrial sequence variation in ancient horses from the Carpathian Basin and possible modern relatives, Genetica 138, 211-218. doi: 10.1007/s10709-009-9411-x.

Pruvost M, Bellone R, Benecke N, Sandoval-Castellanos E, Cieslak M, Kuznetsova T, Morales-Muniz A, O'Connor T, Reissmann M, Hofreiter M and Ludwig A, 2011. Genotypes of predomestic horses match phenotypes painted in Paleolithic works of cave art, Proc Natl Acad Sci U S A 108, 18626-18630. doi: 10.1073/pnas.1108982108.

Pruvost M, Schwarz R, Bessa Correia V, Champlot S, Grange T and Geigl E-M, 2008. DNA diagenesis and palaeogenetic analysis: Critical assessment and methodological progress, Palaeogeogr, Palaeoclimatol, Palaeoecol 266, 211-219. doi: 10.1016/j.palaeo.2008.03.041.

Pruvost M, Schwarz R, Correia VB, Champlot S, Braguier S, Morel N, Fernandez-Jalvo Y, Grange T and Geigl EM, 2007. Freshly excavated fossil bones are best for amplification of ancient DNA, Proc Natl Acad Sci U S A 104, 739-744. doi: 10.1073/pnas.0610257104.

Prystupa JM, Hind P, Cothran EG and Plante Y, 2012. Maternal lineages in native Canadian equine populations and their relationship to the Nordic and Mountain and Moorland pony breeds, J Hered 103, 380-390. doi: 10.1093/jhered/ess003.

R Development Core Team RDCT, 2014. R: a language and environment for statistical computing, R Foundation for Statistical Computing, Vienna.

Rachoud-Schneider A-M, Jacomet S and Zoller H, 1998. Vegetationsveränderungen durch menschlichen Einfluss, Ackerbau, Sammelwirtschaft und Grünlandwirtschaft, in: Hochuli S, Niffeler U and

Rychner V (Eds.), SPM III: Bronzezeit, Verlag Schweizerische Gesellschaft für Ur- und Frühgeschichte, Basel, pp. 151-166.
Rambaut A and Drummond AJ, 2007. Tracer v1.5.
Rawlence NJ, Wood JR, Armstrong KN and Cooper A, 2009. DNA content and distribution in ancient feathers and potential to reconstruct the plumage of extinct avian taxa, Proc Biol Sci 276, 3395-3402. doi: 10.1098/rspb.2009.0755.
Reflexion group on Palaeolithic rock art RgoPra, 1993. L'art pariétal paléolithique, techniques et méthodes d'étude, Edition du Comité des traveaux historiques et scientifiques, Ministère de l'Enseignement Supérieur et de la Recherche, Paris.
Reiche I, Favre-Quattropani L, Vignaud C, Bocherens H, Charlet L and Menu M, 2003. A multi-analytical study of bone diagenesis: the Neolithic site of Bercy (Paris, France), Measurement Science and Technology 14, 1608.
Reissmann M, 2009. Die Farben der Pferde. Genetik, Klassifizierung, Charakteristik, Cadmos, Brunsbek.
Reynolds P, J, 1995. Rural life and farming, in: Green MJ (Ed.), The Celtic world, Routledge, London, pp. 176-210.
Ricaut FX, Keyser-Tracqui C, Crubezy E and Ludes B, 2005. STR-genotyping from human medieval tooth and bone samples, Forensic Sci Int 151, 31-35. doi: 10.1016/j.forsciint.2004.07.001.
Richoz I and Haas JN, 1995. Flora und Vegetation im Schweizer Mittelland und im Jura, in: Stöckli WE, Niffeler U and Gross-Klee E (Eds.), SPM II: Neolithikum, Verlag Schweizerische Gesellschaft für Ur- und Frühgeschichte, Basel, p. 59.
Rieckhoff S and Biel J, 2001. Die Kelten in Deutschland, Wissenschaftliche Buchgesellschaft, Darmstadt.
Rieder S, 2015. Le Haras national suisse à Avenches – centre de l'élevage chevalin, Le chemin et l'histoire 1, 8-14.
Riehl S, Marinova E, Deckers K, Malina M and Conard NJ, 2014. Plant use and local vegetation patterns during the second half of the Late Pleistocene in southwestern Germany, Archeol Anthropol Sci 7, 151-167. doi: 10.1007/s12520-014-0182-7.
Rollo F, Ubaldi M, Marota I, Luciani S and Ermini L, 2002. DNA diagenesis: effect of envinronment and time on human bone, Anc Biomol 4, 1-7.
Rosengren Pielberg G, Golovko A, Sundstrom E, Curik I, Lennartsson J, Seltenhammer MH, Druml T, Binns M, Fitzsimmons C, Lindgren G, Sandberg K, Baumung R, Vetterlein M, Stromberg S, Grabherr M, Wade C, Lindblad-Toh K, Ponten F, Heldin CH, Solkner J and Andersson L, 2008. A cis-acting regulatory mutation causes premature hair graying and susceptibility to melanoma in the horse, Nat Genet 40, 1004-1009. doi: 10.1038/ng.185.
Royo LJ, Alvarez I, Beja-Pereira A, Molina A, Fernandez I, Jordana J, Gomez E, Gutierrez JP and Goyache F, 2005. The origins of Iberian horses assessed via mitochondrial DNA, J Hered 96, 663-669. doi: 10.1093/jhered/esi116.
Sarasin F, Stehlin HG and Studer T, 1918. Die steinzeitlichen Stationen des Birstales zwischen Basel und Delsberg, Neue Denkschriften der Schweizerischen Naturforschenden Gesellschaft 54, 79-290.
Sawyer S, Krause J, Guschanski K, Savolainen V and Pääbo S, 2012. Temporal patterns of nucleotide misincorporations and DNA fragmentation in ancient DNA, PLoS ONE 7, e34131. doi: 10.1371/journal.pone.0034131.
Schaub S and Jagher A, 1945. Zwei neue Fundstellen von Höhlenbär und Höhlenhyäne im unteren Birstal, Ecologae geologicae Helvetiae, 621-634.
Scheu A, Powell A, Bollongino R, Vigne JD, Tresset A, Cakirlar C, Benecke N and Burger J, 2015. The genetic prehistory of domesticated cattle from their origin to the spread across Europe, BMC Genet 16, 54. doi: 10.1186/s12863-015-0203-2.
Schibler J, Elsner J and Schlumbaum A, 2014. Incorporation of aurochs into a cattle herd in Neolithic Europe: single event or breeding?, Sci Rep 4, 5798. doi: 10.1038/srep05798.
Schibler J, Jacomet S, Hüster-Plogmann H and Brombacher C, 1997. Economic crash in the 37th and 36th centuries cal. BC in the Neolithic lake shore sites in Switzerland, Anthropozoologica 25,26, 553-570.

Schlumbaum A, Campos PF, Volken S, Volken M, Hafner A and Schibler J, 2010. Ancient DNA, a Neolithic legging from the Swiss Alps and the early history of goat, J Archaeol Sci 37, 1247-1251. doi: 10.1016/j.jas.2009.12.025.
Schlumbaum A and Edwards CJ, 2013. Ancient DNA research on wetland archaeological evidence, in: Menotti F and O'Sullivan A (Eds.), The Oxford Handbook of Wetland Archaeology, Oxford University Press, Oxford, pp. 569-584.
Schlumbaum A, Neuhaus J-M and Jacomet S, 1998. Coexistence of tetraploid and hexaploid naked wheat in a Neolithic lake dwelling of Central Europe: Evidence from morphology and ancient DNA, J Archaeol Sci 25, 1111-1118. doi: 10.1006/jasc.1998.0338.
Schlumbaum A, Neuhaus JM and Jacomet S, 1997. The prehistoric naked wheat from Swiss lake shore settlements: a study on ancient DNA and morphology, Anc Biomol 1, 244-245.
Schlumbaum A, Stopp B, Breuer G, Rehazek A, Turgay M, Blatter R and Schibler J, 2003. Combining archaeozoology and molecular genetics: the reason behind the changes in cattle size between 150BC and 700AD in Switzerland, Antiquity 77, Project Gallery.
Schlumbaum A, Turgay M and Schibler J, 2006. Near East mtDNA haplotype variants in Roman cattle from Augusta Raurica, Switzerland, and in the Swiss Evolene breed, Anim Genet 37, 373-375. doi: 10.1111/j.1365-2052.2006.01435.x.
Schlumbaum A, van Glabeke S and Roldan-Ruiz I, 2012. Towards the onset of fruit tree growing north of the Alps: ancient DNA from waterlogged apple (*Malus* sp.) seed fragments, Ann Anat 194, 157-162. doi: 10.1016/j.aanat.2011.03.004.
Schlumbaum A and Vandorpe P, 2011. A short history of *Lagenaria siceraria* (bottle gourd) in the Roman provinces: morphotypes and archaeogenetics, Veg Hist Archaeobot 21, 499-509. doi: 10.1007/s00334-011-0343-x.
Schmid E, 1967. Archäologische Bodenforschung des Kantons Basel-Stadt: Riehen-Ausserberg, Basler Zeitschrift für Geschichte und Altertumskunde 67, 30-34.
Schmid E, 1976. Beobachtungen an würmeiszeitlichen Hyänenkoprolithen und zerbissenen Knochen, Thèmes specialises, 143-147.
Schubert M, Jonsson H, Chang D, Der Sarkissian C, Ermini L, Ginolhac A, Albrechtsen A, Dupanloup I, Foucal A, Petersen B, Fumagalli M, Raghavan M, Seguin-Orlando A, Korneliussen TS, Velazquez AM, Stenderup J, Hoover CA, Rubin CJ, Alfarhan AH, Alquraishi SA, Al-Rasheid KA, MacHugh DE, Kalbfleisch T, MacLeod JN, Rubin EM, Sicheritz-Ponten T, Andersson L, Hofreiter M, Marques-Bonet T, Gilbert MT, Nielsen R, Excoffier L, Willerslev E, Shapiro B and Orlando L, 2014. Prehistoric genomes reveal the genetic foundation and cost of horse domestication, Proc Natl Acad Sci U S A 111, E5661-5669. doi: 10.1073/pnas.1416991111.
Schwarz C, Debruyne R, Kuch M, McNally E, Schwarcz H, Aubrey AD, Bada J and Poinar H, 2009. New insights from old bones: DNA preservation and degradation in permafrost preserved mammoth remains, Nucleic Acids Res 37, 3215-3229. doi: 10.1093/nar/gkp159.
Sedlmeier J, 1989. Jungpaläolithikum und Spätpaläolithikum in der Nordwestschweiz. Ein Beitrag zur regionalen Erforschung des Paläolithikums auf Grund ausgewählter Fundinventare aus Grabungen zwischen 1910 und 1956, Bern.
Sedlmeier J, 1993. Steinzeitliche Funde aus der Kohlerhöhle im Laufental, Archäologie Schweiz 16, 40-45.
Sedlmeier J, 2010. Die jungpaläolithischen Funde aus der Mittleren Fundschicht der Kastelhöhle Nord im nordwestschweizerischen Kaltbrunnental, Himmelried SO, Jahrbuch der Archäologie Schweiz 93, 7-34.
Sher AV, 1986. On the history of mammal fauna of Beringida, Quartärpaläontologie 6, 185-193.
Smith CI, Chamberlain AT, Riley MS, Cooper A, Stringer CB and Collins MJ, 2001. Neanderthal DNA. Not just old but old and cold?, Nature 410, 771-772. doi: 10.1038/35071177.
Smith CI, Chamberlain AT, Riley MS, Stringer C and Collins MJ, 2003. The thermal history of human fossils and the likelihood of successful DNA amplification, J Hum Evol 45, 203-217. doi: 10.1016/S0047-2484(03)00106-4.
Sommer RS, Benecke N, Lõugas L, Nelle O and Schmölcke U, 2011. Holocene survival of the wild horse in Europe: a matter of open landscape?, J Quatern Sci 26, 805-812. doi: 10.1002/jqs.1509.

Stampfli HR, Barr JH, Glutz R, Joos M, von Koenigswald W, Lepiksaa J, Müller E, Müller M, Wegmüller S and Wiesli U, 1983. Rislisberghöhle. Archäologie und Ökologie einer Fundstelle aus dem Spätmagdalénien bei Oensingen im Solothurner Jura, Paul Haupt, Bern, Stuttgart.

Stiller M, Baryshnikov G, Bocherens H, Grandal d'Anglade A, Hilpert B, Münzel SC, Pinhasi R, Rabeder G, Rosendahl W, Trinkaus E, Hofreiter M and Knapp M, 2010. Withering away--25,000 years of genetic decline preceded cave bear extinction, Mol Biol Evol 27, 975-978. doi: 10.1093/molbev/msq083.

Stiller M, Molak M, Prost S, Rabeder G, Baryshnikov G, Rosendahl W, Münzel S, Bocherens H, Grandal-d'Anglade A, Hilpert B, Germonpré M, Stasyk O, Pinhasi R, Tintori A, Rohland N, Mohandesan E, Ho SYW, Hofreiter M and Knapp M, 2014. Mitochondrial DNA diversity and evolution of the Pleistocene cave bear complex, Quatern Int 339-340, 224-231. doi: 10.1016/j.quaint.2013.09.023.

Svensson EM, Hasler S, Nussbaumer M, Rehazek A, Omrak A and Götherström A, 2014. Medieval cattle from Bern (Switzerland): an archaeozoological, genetic and historical approach, Schweiz Arch Tierheilkd 156, 17-26. doi: 10.1024/0036-7281/a000542.

Svensson EM, Telldahl Y, Sjoling E, Sundkvist A, Hulth H, Sjovold T and Götherström A, 2012. Coat colour and sex identification in horses from Iron Age Sweden, Ann Anat 194, 82-87. doi: 10.1016/j.aanat.2011.11.001.

Tamura K and Nei M, 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees, Mol Biol Evol 10, 512-526. doi: 10.1093/oxfordjournals.molbev.a040023.

Terberger T and Street M, 2001. Hiatus or continuity? New results for the question of peniglacial settlement in Europe, Antiquity 76, 691-698.

Tillet T, 2001. Les Alpes et le Jura: quaternaire et préhistorique ancienne, Paris.

Troy CS, MacHugh DE, Bailey JF, Magee DA, Loftus RT, Cunningham P, Chamberlain AT, Sykes BC and Bradley DG, 2001. Genetic evidence for Near-Eastern origins of European cattle, Nature 410, 1088-1091. doi: 10.1038/35074088.

Trut L, Oskina I and Kharlamova A, 2009. Animal evolution during domestication: the domesticated fox as a model, Bioessays 31, 349-360. doi: 10.1002/bies.200800070.

Vila C, Leonard JA, Götherström A, Marklund S, Sandberg K, Liden K, Wayne RK and Ellegren H, 2001. Widespread origins of domestic horse lineages, Science 291, 474-477. doi: 10.1126/science.291.5503.474.

Vilstrup JT, Seguin-Orlando A, Stiller M, Ginolhac A, Raghavan M, Nielsen SC, Weinstock J, Froese D, Vasiliev SK, Ovodov ND, Clary J, Helgen KM, Fleischer RC, Cooper A, Shapiro B and Orlando L, 2013. Mitochondrial phylogenomics of modern and ancient equids, PLoS ONE 8, e55950. doi: 10.1371/journal.pone.0055950.

Vives S, Gilbert MT, Arenas C, Gigli E, Lao O and Lalueza-Fox C, 2008. Statistical analysis of post mortem DNA damage-derived miscoding lesions in Neandertal mitochondrial DNA, BMC Res Notes 1, 40. doi: 10.1186/1756-0500-1-40.

Vogt E, 1936. Die paläolithische Station in der Höhle am Schalbergfelsen, Denkschriften der Schweizerischen Naturforschenden Gesellschaft 71, 1-70.

Warmuth V, Eriksson A, Bower MA, Barker G, Barrett E, Hanks BK, Li S, Lomitashvili D, Ochir-Goryaeva M, Sizonov GV, Soyonov V and Manica A, 2012. Reconstructing the origin and spread of horse domestication in the Eurasian steppe, Proc Natl Acad Sci U S A 109, 8202-8206. doi: 10.1073/pnas.1111122109.

Warmuth V, Eriksson A, Bower MA, Canon J, Cothran G, Distl O, Glowatzki-Mullis ML, Hunt H, Luis C, do Mar Oom M, Yupanqui IT, Zabek T and Manica A, 2011. European domestic horses originated in two holocene refugia, PLoS ONE 6, e18194. doi: 10.1371/journal.pone.0018194.

Warnberg O and Alt KW, 2012. Molekulargenetische Analysen an den Bestattungen aus dem endneolithischen Kollektivgrab von Spreitenbach, in: Doppler T (Ed.), Spreitenbach-Moosweg (Aargau, Schweiz): ein Kollektivgrab um 2500 v. Chr., Archäologie Schweiz, Basel, pp. 158-169.

Weinstock J, Willerslev E, Sher A, Tong W, Ho SY, Rubenstein D, Storer J, Burns J, Martin L, Bravi C, Prieto A, Froese D, Scott E, Xulong L and Cooper A, 2005. Evolution, systematics, and

phylogeography of pleistocene horses in the new world: a molecular perspective, PLoS Biol 3, e241. doi: 10.1371/journal.pbio.0030241.
Welch AJ, Wiley AE, James HF, Ostrom PH, Stafford TW, Jr. and Fleischer RC, 2012. Ancient DNA reveals genetic stability despite demographic decline: 3,000 years of population history in the endemic Hawaiian petrel, Mol Biol Evol 29, 3729-3740. doi: 10.1093/molbev/mss185.
Wick L, 2002. Die natürliche Vegetation, in: Flutsch L, Niffeler U and Rossi F (Eds.), SPM V: Römische Zeit, Verlag Schweizerische Gesellschaft für Ur- und Frühgeschichte, Basel, pp. 32-33.
Wick L, Jacomet S, Schlumbaum A and Schibler J, 2002. Menschlicher Einfluss auf die natürliche Vegetation, in: Flutsch L, Niffeler U and Rossi F (Eds.), SPM V: Römische Zeit, Verlag Schweizerische Gesellschaft für Ur- und Frühgeschichte, Basel, pp. 34-36.
Willerslev E, Cappellini E, Boomsma W, Nielsen R, Hebsgaard MB, Brand TB, Hofreiter M, Bunce M, Poinar HN, Dahl-Jensen D, Johnsen S, Steffensen JP, Bennike O, Schwenninger JL, Nathan R, Armitage S, de Hoog CJ, Alfimov V, Christl M, Beer J, Muscheler R, Barker J, Sharp M, Penkman KE, Haile J, Taberlet P, Gilbert MT, Casoli A, Campani E and Collins MJ, 2007. Ancient biomolecules from deep ice cores reveal a forested southern Greenland, Science 317, 111-114. doi: 10.1126/science.1141758.
Willerslev E, Gilbert MT, Binladen J, Ho SY, Campos PF, Ratan A, Tomsho LP, da Fonseca RR, Sher A, Kuznetsova TV, Nowak-Kemp M, Roth TL, Miller W and Schuster SC, 2009. Analysis of complete mitochondrial genomes from extinct and extant rhinoceroses reveals lack of phylogenetic resolution, BMC Evol Biol 9, 95. doi: 10.1186/1471-2148-9-95.
Xu X and Arnason U, 1994. The complete mitochondrial DNA sequence of the horse, *Equus caballus*: extensive heteroplasmy of the control region, Gene 148, 357-362.
Zouganelis GD, Ogden R, Nahar N, Runfola V, Bonab M, Ardalan A, Radford D, Barnett R, Larson G, Hildred A, Jones M and Scarlett G, 2014. An old dog and new tricks: Genetic analysis of a Tudor dog recovered from the Mary Rose wreck, Forensic Sci Int 245, 51-57. doi: 10.1016/j.forsciint.2014.10.001.
Zuberbühler Koch K, 2002. Die magdalénienzeitliche Höhlenstation Winznau/Käsloch im Kanton Solothurn, Bern.

Eidesstattliche Erklärung

Hiermit versichere ich, dass ich die Dissertation selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe, alle Ausführungen, die anderen Schriften wörtlich oder sinngemäß entnommen wurden, kenntlich gemacht sind und die Arbeit in gleicher oder ähnlicher Fassung noch nicht Bestandteil einer Studien- oder Prüfungsleistung war.

Julia Elsner